JUL 21 1924

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
PERISTALTIC RUSH IN THE RABBIT. *Walter C. Alvarez and Lucille J. Mahoney*	211
PERISTALTIC RUSH AS DEPICTED IN THE ELECTROENTEROGRAM. *Walter C. Alvarez and Lucille J. Mahoney*	226
BAYLISS AND STARLING'S LAW OF THE INTESTINE, OR, THE MYENTERIC REFLEX. *Walter C. Alvarez*	229
A NON-POLARIZABLE ELECTRODE AND CLAMP DESIGNED PARTICULARLY FOR GASTRO-INTESTINAL WORK. *Walter C. Alvarez*	249
A PHYSIOLOGICAL STUDY OF THE REACTIONS OF YOUNG DECEREBRATE ANIMALS. *Orthello R. Langworthy*	254
REACTIONS OF INTESTINAL SEGMENTS TO MECHANICAL STIMULATION. *R. G. Hoskins and Edgar S. Hunter*	265
THE EFFECT OF INGESTED ADRENALIN CHLORIDE ON BASAL METABOLISM. *F. A. Hitchcock*	271
STUDIES ON THE LOCOMOTION OF LEUCOCYTES. III. THE RATE OF LOCOMOTION OF HUMAN LYMPHOCYTES IN VITRO. *Morton McCutcheon*	279
ELECTRICAL RECORDS OF AFFERENT NERVE IMPULSES FROM MUSCULAR RECEPTORS. *A. Forbes, C. J. Campbell and H. B. Williams*	283
THE EFFECT OF DIFFERENT ANESTHETICS ON THE CATALASE CONTENT AND OXYGEN CONSUMPTION OF UNICELLULAR ORGANISMS. *W. E. Burge*	304
THE PERIPHERAL WATER LOSS IN RABBITS AS A FACTOR IN HEAT REGULATION. *Robert W. Keeton*	307
ON THE RELATIONSHIP BETWEEN THE GASTRIC ACID RESPONSE AND THE BASAL SECRETION OF THE STOMACH. *Robert K. S. Lim*	318
STUDIES IN BLOOD DIASTASE. II. ARE THE BLOOD DIASTASES END PRODUCTS OF METABOLISM? *Seymour J. Cohen*	334
COLOR PHENOMENA CAUSED BY INTERMITTENT STIMULATION WITH WHITE LIGHT. *G. N. Stewart*	337
SECONDARY EXCITATION IN THE RETINA. *William R. Amberson*	354
STUDIES ON THE PHYSIOLOGY OF THE LIVER. VIII. EFFECT OF TOTAL REMOVAL OF THE LIVER ON THE FORMATION OF UREA. *Jesse L. Bollman, Frank C. Mann and Thomas B. Magath*	371
STUDIES ON THE PHYSIOLOGY OF THE LIVER. IX. THE FORMATION OF BILE PIGMENT AFTER TOTAL REMOVAL OF THE LIVER. *Frank C. Mann, Jesse L. Bollman and Thomas B. Magath*	393
CIRCULATORY RESPONSES TO EXERCISE IN MAN AND THEIR BEARINGS ON THE QUESTION OF DIASTOLIC HEART TONE. *H. L. White*	410
THE ACTION OF PREPARATIONS OF THE ENDOCRINE GLANDS UPON THE WORK DONE BY SKELETAL MUSCLE. *Nathan B. Eddy*	432
THE PHYSIOLOGICAL MAXIMUM RATE OF THE HEART. *Joseph Tulgan*	441
THE EFFECT OF ADRENALIN UPON THE PATELLAR TENDON REFLEX. *W. W. Tuttle*	446

VOL. LXIX—NO. 2

Issued July 1, 1924

BALTIMORE, U. S. A.

1924

d as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

MERCURY MANOMETER WITH THREE-WAY COCK

THIS manometer is constructed of carefully selected calibrated, annealed glass tubing, which is mounted on a hard rubber block. The horse-hair and weight of the actual manometer are replaced by a slotted brass tube which serves as a guide for the writing needle, assuring continuous approximation of writing point to paper. The writing point is brought into contact with the paper by turning a screw which rotates the brass guide tube. The scale is not fixed, as in other types of manometer, but can be adjusted at "blood pressure zero" for each experiment. The three-way cock serves to put into communication the anti-clot reservoir, the manometer and the blood vessel.

PRICE $23.00

Catalogue on Request

JOSEPH BECKER

437 WEST 59TH STREET **NEW YORK**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 69 JULY 1, 1924 No. 2

PERISTALTIC RUSH IN THE RABBIT

WALTER C. ALVAREZ AND LUCILLE J. MAHONEY

From the George Williams Hooper Foundation for Medical Research and the Department of Medicine, University of California Medical School, San Francisco

Received for publication March 29, 1924

As soon as physiologists began to study the movements of the intestine with the animal opened under salt solution, they observed that there are two main types of activity. In the first place, there are small localized rhythmic contractions which do not seem to forward the intestinal contents to any great extent but which knead those contents; mix them with the digestive juices, and spread them again and again over the absorbing surface of the mucous membrane. Then, from time to time, there appear larger waves which usually run from one end of the bowel to the other and which serve to carry the food on its way from the stomach to the rectum. They have been called by different writers peristaltic rushes, diastaltic waves, rollbewegungen and schubbewegungen. On account of their important function in health and disease, these waves must always be of the greatest interest to the physiologist, the physician and the surgeon, and it is unfortunate that as yet we know so little about their origin and their mode of propagation.

Probably the best article on the subject, and the only one we have found dealing exclusively with it, is that by Meltzer and Auer (1), who introduced the term "peristaltic rush." They reviewed much of the early literature and then described their own experiments, most of which were concerned with the production of rush waves by means of drugs. The great defect in this and in all other articles which we have found touching upon the subject is the lack of graphic records showing the rushes passing a number of points on the bowel. The trouble has been, of course, that there are technical difficulties in the way, but these have now, to a large extent, been overcome. It can easily be seen that little exact information

could be obtained about the movements of the digestive tract so long as observers contented themselves with trying to watch what goes on at one time in the many different parts of a tube several meters in length. Not only do too many things happen at once, but many of the slow changes in tone and the smaller rhythmic movements cannot be seen until they are enlarged and recorded by appropriate devices.

Technic. In 1913 the senior writer devised a little enterograph with the aid of which he was able to get simultaneous records from many parts of the digestive tracts of laboratory animals; and a number of good records of peristaltic rushes were then obtained (2). Unfortunately, the amplitude of the movements at the recording end was rather small, and the apparatus generally failed to show the tonus changes which are so interesting and which probably have a large significance. Later, an "L" recorder was devised. (See fig. 3 in an article by Alvarez and Mahoney (3).) This consisted of a brass rod with a little wire serrefine at the lower end. The serrefine served to fix one point on the surface of the bowel. Another point 2 cm. away was pinched lightly by another serrefine on the end of a thread which ran over a tiny pulley at the outer end of the horizontal bar of the L, and up to a light heart lever writing on a kymograph. As the bowel contracted, the recording point of the lever would write upwards on the drum. Fair records were obtained with this instrument but the pulley caused so much friction that it had to be eliminated.

During the last two years we have been getting very satisfactory records in the following way. From six to eight places on the stomach or bowel are seized by little serrefines or spring clips 16 mm. long, which we make out of piano wire. These little clips which are fastened to the ends of the horizontal arms of "L" rods, serve to hold the several segments in place under the bath of warm Locke's solution. Other similar serrefines are attached nearby in such a way that either the circular or the longitudinal fibers (as desired) will pull on threads which run directly up to light levers recording on a large kymograph drum. The kymograph is placed on a shelf which extends out over the bath in which the animal lies: anesthetized and with its abdomen open. Urethane is used as an anesthetic and, in addition, the spinal cord is always destroyed as far up as the shoulder region.

In all tracings the uppermost record is from the most orad segment used, and the rest follow seriatim. An upstroke indicates contraction of the bowel. The use of circular or longitudinal muscle will be noted on the margin, as will be also the distance from the pylorus. As the bowel loses its tone and changes its length markedly after death, these distances are measured at the close of the day before the animal is killed. A knotted string is used as a tape measure and the figures can only be approximate. The time record indicates ten-second intervals. The magnification pro-

duced by the levers is 4 ×. In some records there is a signal mark noting the time of passage of the rush under certain recorders numbered from above downwards. Small marks have been made to show synchronous points.

The best results have been obtained with rabbits, and almost all the statements made in this article are based on work done with them. Many of these animals show good rhythmic movements and good spontaneous rushes shortly after they are opened. Others, perhaps on account of the presence of parasites or disease, are unsatisfactory. We have had the same difficulties that other observers have had in trying to evoke peristalsis in cats. Bayliss and Starling and Cannon gave purgatives the night before, but we have been very reluctant to do that since Alvarez and Taylor (4) found how toxic such purged bowels can be. Satisfactory records can be obtained in many animals only by waiting for several hours. Some of the shock of the operative procedures may then wear off and, in addition, there probably is some stimulus to peristalsis derived from the exposure to the bath and from the handling of the bowel.

We have felt that the first thing to do with a phenomenon such as peristaltic rush is to watch it and study it as it occurs spontaneously, and under conditions as nearly normal as possible. Later, when we have learned all we can in this way, we can more safely begin to experiment. Most of the observers who have studied this problem in the past have inserted balloons or boluses of cotton or food through holes made in the bowel, but we have thought it best, at least at first, to study the way in which the bowel handles the material which comes to it normally from the stomach. In many instances we have injected water into the esophagus, and at other times we have injected water or air into the bowel through a hypodermic needle. Although the serrefines pinch only a very small area on the peritoneal surface of the bowel and ordinarily do not traumatize it very much, we have some evidence which makes us feel that at times their presence does somewhat hamper the progress of material through the small bowel. This is due perhaps to the kinks which are produced by binding down parts of the bowel, or possibly more often to the traumatization of certain regions which makes them more irritable and thus changes the gradient down the bowel (5).

Rhythmic segmentation. The rhythmic segmentation so commonly observed in the upper small bowel of cats has been so well described by Cannon (6) that we will make no comment upon it. In rabbits one sees more often the pendulum movements in which contractions occur at the two ends of a short loop and throw the contents backwards and forwards. There seems to be little difference between the two mechanisms except that in the latter case the distance between the two contracting places is a little longer. It is this to and fro movement which makes the small,

regular contractions on our records. The rate varies inversely as the distance from the pylorus. These rhythmic kneading movements may contribute a little toward the forwarding of the intestinal contents because the contraction at the upper end of a loop comes a little more frequently than at the lower; it seems often to be more forceful, and it may cause some of the material in the loop to trickle past the lower end.

Origin of the rushes. In 1913, as soon as the first graphic records of rushes were obtained, it was noted that most of them seem to arise in the duodenum about the time that a gastric wave reaches the pylorus. Sometimes, as in figure 1 A, this wave is a particularly powerful one which comes perhaps at the end of a series of waves of increasing amplitude, and the stomach seems to make a special effort to start the rush. At other times, as in figure 1 B, there is nothing peculiar about the gastric wave just before the rush, or there may be no definite wave at all, as in figure 1 C. See also figures 2 A, 3 B, 4 A and 4 C in the Myenteric Reflex paper following.

It is interesting to note in many of the records, as in figure 1 A, that after starting a big rush the stomach seems to be fatigued or inhibited for a while. At times this is definitely due to inhibition because it occurs when the rush has started low down in the bowel, a long way from the stomach.

It seems pretty clear from our observations that the stomach does not have to empty any of its contents into the duodenum in order to start a rush. In fact, when we came to make a more careful study of the time relations between the gastric waves and the duodenal tonus contractions, from which the rush waves seem to originate, we found that these tonus waves come a few seconds *before* the gastric waves reach the pylorus (7). The senior writer has made this observation time and again while screening men and women after barium meals, and Pirie showed the phenomenon very clearly in serial roentgenograms demonstrated at the 1922 meeting of the American Roentgen Ray Society. Apparently some influence travels out ahead of the visible wave in the antrum because there is an emptying of the distal half of the duodenal cap when the crest of the gastric wave is still two or three centimeters above the pylorus. Sometimes, with a very irritable digestive tract, a second rush will go off down the bowel a few seconds later, when the stomach discharges some of its contents into the duodenum. Perhaps this mechanism can account for the double rushes seen in some of the records, as in figure 3 C. Occasionally in man the stomach empties with a wave which seems to run from the antrum right on down the duodenum. In rabbits the duodenum is often filled from the stomach without the production of any rush down the bowel.

The next question is: May not some of the rushes begin in regions even orad to the stomach? This seems to be the case because they so often

depart about the time the animal swallows. In fact, we have found that the easiest way in which to start rushes is to bring about swallowing by injecting a little water into the pharynx., The passage of the water down into the stomach and bowel helps to start the rushes, but it is not essential because they will start sometimes also when the swallowing is produced simply by tickling the fauces, or by stimulating the upper esophagus electrically. These findings may perhaps be of interest to the surgeon who is often very anxious to start up peristalsis after abdominal operations. Actually some surgeons do find it a good thing to let their patients drink water freely even on the first day after laparotomies.

Another way in which rush waves can be started is by stimulating the duodenum. At times, after this part of the bowel has been segmenting actively for a while, it seems to get on a hair trigger and a slight pinch or series of pinches will be sure to start a rush. After that, for some time no more rushes can be started, and the bowel is apparently fatigued or refractory. The former term is perhaps the more correct because we see occasionally, as in figure 3 C, that one rush can follow very closely on another, and it is a question whether or not a true refractory state ever exists.

The records show clearly that rushes can begin anywhere in the bowel. What happens usually is that a loop fills slowly from above, as will be seen in figure 4. That group of tracings shows, incidentally, how liquids can seep from one end of the tract to the other without the help of peristaltic rushes. The presence of material in the loops causes their tone to rise and the amplitude of their contractions to increase. Material is thrown backwards and forwards by the two ends of the loop until the upper end seems to overcome the lower, and then down goes a rush. Often, as in figure 2 B, the bowel nearly produces a rush from time to time but it does not quite succeed. Especially in the rather insensitive ileum, after one or two such attempts at starting a rush, the bowel will often give up and will quiet down. *At other times a ripple will come down from the stomach* and that will apparently give the necessary impetus to start the rush; or again, the necessary disturbance in the equilibrium of the bowel will come through the emptying of a loop somewhere along the line, or from a defecation.

After the loop empties, its tone drops, as can be seen in figures 1 A, 2 A and 4, and the rhythmic contractions may almost cease, as in figure 2 C. When the amplitude of these contractions drops for a moment and is then restored we can be pretty sure that the rush which passed did not carry forward much of the material, or if it did, much more new material came down from above immediately afterwards. (See figs. 3 A and 3 C.)

Usually during digestion a number of loops from 10 to 50 cm. apart are filled and active, and when a rush comes, the material in the loops moves

ERRATUM

In Volume 69, no. 2, July, 1924

Figure on p. 448 should be figure 2.
Figure on p. 451 should be figure 1.

XUM

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 69 JULY 1, 1924 No. 2

PERISTALTIC RUSH IN THE RABBIT

WALTER C. ALVAREZ AND LUCILLE J. MAHONEY

From the George Williams Hooper Foundation for Medical Research and the Department of Medicine, University of California Medical School, San Francisco

Received for publication March 29, 1924

As soon as physiologists began to study the movements of the intestine with the animal opened under salt solution, they observed that there are two main types of activity. In the first place, there are small localized rhythmic contractions which do not seem to forward the intestinal contents to any great extent but which knead those contents; mix them with the digestive juices, and spread them again and again over the absorbing surface of the mucous membrane. Then, from time to time, there appear larger waves which usually run from one end of the bowel to the other and which serve to carry the food on its way from the stomach to the rectum. They have been called by different writers peristaltic rushes, diastaltic waves, rollbewegungen and schubbewegungen. On account of their important function in health and disease, these waves must always be of the greatest interest to the physiologist, the physician and the surgeon, and it is unfortunate that as yet we know so little about their origin and their mode of propagation.

Probably the best article on the subject, and the only one we have found dealing exclusively with it, is that by Meltzer and Auer (1), who introduced the term "peristaltic rush." They reviewed much of the early literature and then described their own experiments, most of which were concerned with the production of rush waves by means of drugs. The great defect in this and in all other articles which we have found touching upon the subject is the lack of graphic records showing the rushes passing a number of points on the bowel. The trouble has been, of course, that there are technical difficulties in the way, but these have now, to a large extent, been overcome. It can easily be seen that little exact information

could be obtained about the movements of the digestive tract so long as observers contented themselves with trying to watch what goes on at one time in the many different parts of a tube several meters in length. Not only do too many things happen at once, but many of the slow changes in tone and the smaller rhythmic movements cannot be seen until they are enlarged and recorded by appropriate devices.

Technic. In 1913 the senior writer devised a little enterograph with the aid of which he was able to get simultaneous records from many parts of the digestive tracts of laboratory animals; and a number of good records of peristaltic rushes were then obtained (2). Unfortunately, the amplitude of the movements at the recording end was rather small, and the apparatus generally failed to show the tonus changes which are so interesting and which probably have a large significance. Later, an "L" recorder was devised. (See fig. 3 in an article by Alvarez and Mahoney (3).) This consisted of a brass rod with a little wire serrefine at the lower end. The serrefine served to fix one point on the surface of the bowel. Another point 2 cm. away was pinched lightly by another serrefine on the end of a thread which ran over a tiny pulley at the outer end of the horizontal bar of the L, and up to a light heart lever writing on a kymograph. As the bowel contracted, the recording point of the lever would write upwards on the drum. Fair records were obtained with this instrument but the pulley caused so much friction that it had to be eliminated.

During the last two years we have been getting very satisfactory records in the following way. From six to eight places on the stomach or bowel are seized by little serrefines or spring clips 16 mm. long, which we make out of piano wire. These little clips which are fastened to the ends of the horizontal arms of "L" rods, serve to hold the several segments in place under the bath of warm Locke's solution. Other similar serrefines are attached nearby in such a way that either the circular or the longitudinal fibers (as desired) will pull on threads which run directly up to light levers recording on a large kymograph drum. The kymograph is placed on a shelf which extends out over the bath in which the animal lies: anesthetized and with its abdomen open. Urethane is used as an anesthetic and, in addition, the spinal cord is always destroyed as far up as the shoulder region.

In all tracings the uppermost record is from the most orad segment used, and the rest follow seriatim. An upstroke indicates contraction of the bowel. The use of circular or longitudinal muscle will be noted on the margin, as will be also the distance from the pylorus. As the bowel loses its tone and changes its length markedly after death, these distances are measured at the close of the day before the animal is killed. A knotted string is used as a tape measure and the figures can only be approximate. The time record indicates ten-second intervals. The magnification pro-

duced by the levers is 4 ×. In some records there is a signal mark noting the time of passage of the rush under certain recorders numbered from above downwards. Small marks have been made to show synchronous points.

The best results have been obtained with rabbits, and almost all the statements made in this article are based on work done with them. Many of these animals show good rhythmic movements and good spontaneous rushes shortly after they are opened. Others, perhaps on account of the presence of parasites or disease, are unsatisfactory. We have had the same difficulties that other observers have had in trying to evoke peristalsis in cats. Bayliss and Starling and Cannon gave purgatives the night before, but we have been very reluctant to do that since Alvarez and Taylor (4) found how toxic such purged bowels can be. Satisfactory records can be obtained in many animals only by waiting for several hours. Some of the shock of the operative procedures may then wear off and, in addition, there probably is some stimulus to peristalsis derived from the exposure to the bath and from the handling of the bowel.

We have felt that the first thing to do with a phenomenon such as peristaltic rush is to watch it and study it as it occurs spontaneously, and under conditions as nearly normal as possible. Later, when we have learned all we can in this way, we can more safely begin to experiment. Most of the observers who have studied this problem in the past have inserted balloons or boluses of cotton or food through holes made in the bowel, but we have thought it best, at least at first, to study the way in which the bowel handles the material which comes to it normally from the stomach. In many instances we have injected water into the esophagus, and at other times we have injected water or air into the bowel through a hypodermic needle. Although the serrefines pinch only a very small area on the peritoneal surface of the bowel and ordinarily do not traumatize it very much, we have some evidence which makes us feel that at times their presence does somewhat hamper the progress of material through the small bowel. This is due perhaps to the kinks which are produced by binding down parts of the bowel, or possibly more often to the traumatization of certain regions which makes them more irritable and thus changes the gradient down the bowel (5).

Rhythmic segmentation. The rhythmic segmentation so commonly observed in the upper small bowel of cats has been so well described by Cannon (6) that we will make no comment upon it. In rabbits one sees more often the pendulum movements in which contractions occur at the two ends of a short loop and throw the contents backwards and forwards. There seems to be little difference between the two mechanisms except that in the latter case the distance between the two contracting places is a little longer. It is this to and fro movement which makes the small,

regular contractions on our records. The rate varies inversely as the distance from the pylorus. These rhythmic kneading movements may contribute a little toward the forwarding of the intestinal contents because the contraction at the upper end of a loop comes a little more frequently than at the lower; it seems often to be more forceful, and it may cause some of the material in the loop to trickle past the lower end.

Origin of the rushes. In 1913, as soon as the first graphic records of rushes were obtained, it was noted that most of them seem to arise in the duodenum about the time that a gastric wave reaches the pylorus. Sometimes, as in figure 1 A, this wave is a particularly powerful one which comes perhaps at the end of a series of waves of increasing amplitude, and the stomach seems to make a special effort to start the rush. At other times, as in figure 1 B, there is nothing peculiar about the gastric wave just before the rush, or there may be no definite wave at all, as in figure 1 C. See also figures 2 A, 3 B, 4 A and 4 C in the Myenteric Reflex paper following.

It is interesting to note in many of the records, as in figure 1 A, that after starting a big rush the stomach seems to be fatigued or inhibited for a while. At times this is definitely due to inhibition because it occurs when the rush has started low down in the bowel, a long way from the stomach.

It seems pretty clear from our observations that the stomach does not have to empty any of its contents into the duodenum in order to start a rush. In fact, when we came to make a more careful study of the time relations between the gastric waves and the duodenal tonus contractions, from which the rush waves seem to originate, we found that these tonus waves come a few seconds *before* the gastric waves reach the pylorus (7). The senior writer has made this observation time and again while screening men and women after barium meals, and Pirie showed the phenomenon very clearly in serial roentgenograms demonstrated at the 1922 meeting of the American Roentgen Ray Society. Apparently some influence travels out ahead of the visible wave in the antrum because there is an emptying of the distal half of the duodenal cap when the crest of the gastric wave is still two or three centimeters above the pylorus. Sometimes, with a very irritable digestive tract, a second rush will go off down the bowel a few seconds later, when the stomach discharges some of its contents into the duodenum. Perhaps this mechanism can account for the double rushes seen in some of the records, as in figure 3 C. Occasionally in man the stomach empties with a wave which seems to run from the antrum right on down the duodenum. In rabbits the duodenum is often filled from the stomach without the production of any rush down the bowel.

The next question is: May not some of the rushes begin in regions even orad to the stomach? This seems to be the case because they so often

depart about the time the animal swallows. In fact, we have found that the easiest way in which to start rushes is to bring about swallowing by injecting a little water into the pharynx. The passage of the water down into the stomach and bowel helps to start the rushes, but it is not essential because they will start sometimes also when the swallowing is produced simply by tickling the fauces, or by stimulating the upper esophagus electrically. These findings may perhaps be of interest to the surgeon who is often very anxious to start up peristalsis after abdominal operations. Actually some surgeons do find it a good thing to let their patients drink water freely even on the first day after laparotomies.

Another way in which rush waves can be started is by stimulating the duodenum. At times, after this part of the bowel has been segmenting actively for a while, it seems to get on a hair trigger and a slight pinch or series of pinches will be sure to start a rush. After that, for some time no more rushes can be started, and the bowel is apparently fatigued or refractory. The former term is perhaps the more correct because we see occasionally, as in figure 3 C, that one rush can follow very closely on another, and it is a question whether or not a true refractory state ever exists.

The records show clearly that rushes can begin anywhere in the bowel. What happens usually is that a loop fills slowly from above, as will be seen in figure 4. That group of tracings shows, incidentally, how liquids can seep from one end of the tract to the other without the help of peristaltic rushes. The presence of material in the loops causes their tone to rise and the amplitude of their contractions to increase. Material is thrown backwards and forwards by the two ends of the loop until the upper end seems to overcome the lower, and then down goes a rush. Often, as in figure 2 B, the bowel nearly produces a rush from time to time but it does not quite succeed. Especially in the rather insensitive ileum, after one or two such attempts at starting a rush, the bowel will often give up and will quiet down. At other times a ripple will come down from the stomach and that will apparently give the necessary impetus to start the rush; or again, the necessary disturbance in the equilibrium of the bowel will come through the emptying of a loop somewhere along the line, or from a defecation.

After the loop empties, its tone drops, as can be seen in figures 1 A, 2 A and 4, and the rhythmic contractions may almost cease, as in figure 2 C. When the amplitude of these contractions drops for a moment and is then restored we can be pretty sure that the rush which passed did not carry forward much of the material, or if it did, much more new material came down from above immediately afterwards. (See figs. 3 A and 3 C.)

Usually during digestion a number of loops from 10 to 50 cm. apart are filled and active, and when a rush comes, the material in the loops moves

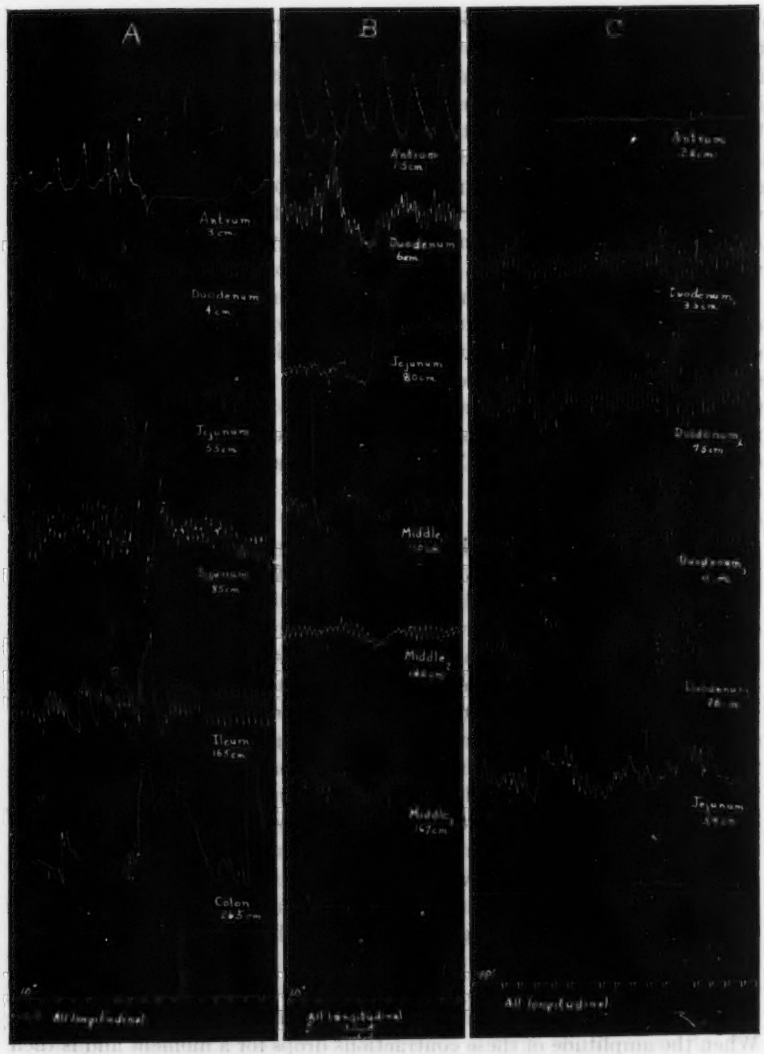


Fig. 1. Shows peristaltic rushes in the rabbit.

A. Shows a rush from stomach to colon followed by fatigue (?) in the stomach and deep drops in tone in the bowel.

B. Shows no change in the gastric record with the rush. This rush faded out in the upper ileum.

C. Shows how one rush can be associated with a gastric wave and the next one may originate elsewhere.

down one or two steps much as the players shift on the diamond when the bases are full. Just as in the baseball game, the shift is due generally to a new player arriving on first base, but it seems also to be due sometimes to some one going "home," i.e., material passing out through the ileocecal or anal sphincters.

We have noticed quite often that when some disturbance is passing along the bowel, a rise in tone will take place almost simultaneously in many parts of the tract. This sort of thing may be seen in several of the figures published here. Thus in figure 1 A the rise in tone of the colon is practically synchronous with the beginning of the rush in the stomach and duodenum. Similarly in figure 2 B the rise in the tone of the colon probably began about the time that the rush which appears in the ileal records left the stomach or duodenum. In figure 2 C we find more evidence of a disturbance beginning about the same time in the upper abdomen and the terminal ileum. These disturbances probably travel by way of the nerves either in the bowel wall or in the mesentery. They jump some regions to appear in others; and they probably help to keep the digestive tract acting as a unit.

Perhaps most of the rush waves in the rabbit's small intestine stop in the lower ileum but some go on down the colon, and on their way they not infrequently produce contractions of the cecum. In a few rabbits we have, by repeated stimulation of the duodenum, been able to produce so many complete rushes as almost to empty the bowel. The mass movements in the colon of man probably correspond to the rushes in the small bowel, and there is evidence which suggests that they often originate in ripples coming from the upper part of the tract.

When the rush waves stop in the lower ileum they either fade out and seem to lose themselves in this more sluggish part of the bowel or else they are met by powerful contractions and a marked rise of tone. (See figs. 1 and 2 A in the following paper on the Law of the Intestine.) It is fortunate for us that so many rushes are stopped in this way or also we would soon die of starvation and diarrhea. It has long been suspected by some writers that the muscle near the ileocecal sphincter serves not only to prevent regurgitation but to prevent too rapid emptying downward of the ileum, and we now have much evidence to show that they are right.

Influence on the brain. An interesting feature about the rushes is that they seem to affect the animal's brain quite markedly, even when it is well under the anesthetic, and when the only path from the bowel is along the vagi. Often as the rush begins, or as it proceeds down the bowel, there is a deep sigh, and not infrequently a slight struggle with the forepaws (the hind ones are cut off by the destruction of the cord), as if the animal were going to be roused from the anesthetic. (See figs. 2 B, 2 C and 3 C.) This is of some interest because men and women are some-

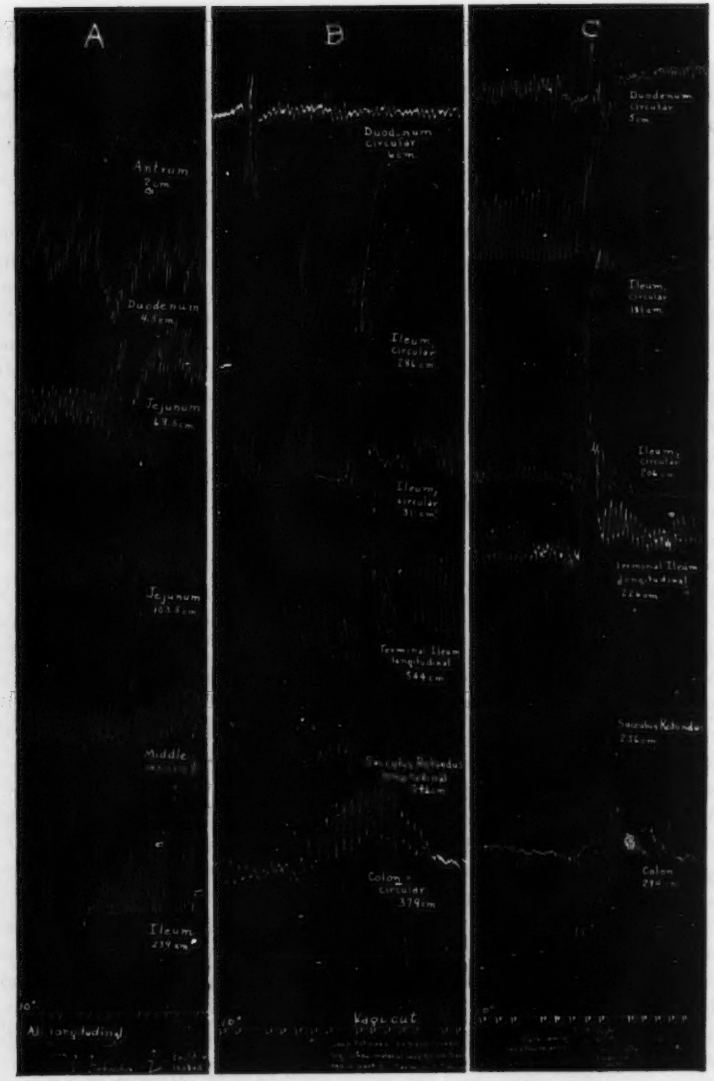


Fig. 2. A. A rush followed defecation which may have caused the temporary inhibition of the stomach and probably also the rush. Note the big drop in duodenal tone after the rush. The big rise in the jejunum after the rush means generally that considerable material has been left behind there.

B. Shows big tonus changes and attempts at rush in the ileum. The big disturbance in the duodenum was due to some deep sighs. Note the big rise in the colon starting about the time the rush left the upper bowel.

C. Shows the drop in tone and activity of the ileum after a rush has emptied it of its contents. Note sigh as rush starts.

times greatly distressed and frightened by their rush waves. They feel queer; sweat may break out on them and occasionally they faint at the toilet. Ordinarily, however, the rushes do not seem to bother us in health because often, with the roentgen ray, the senior writer has watched them whisking down the jejunum without affecting in any way the consciousness of the patient.

We made some studies to see just when the animals are upset by the rush and found that the sigh or struggle comes most often when the preliminary gastric wave reaches the preantrum; that is, just before the rush starts off down the bowel. As we should expect, this tendency of the animal to struggle reflexly as the wave goes down the bowel is apparently removed by cutting the vagi just below the diaphragm. In one animal out of the four operated upon in this way there was one instance in which a deep sigh was coincident with a rush, but this may perhaps have been accidental or, more probably, the wave may have originated in the pharynx from whence it could affect the sensorium.

Rate of travel. We have been handicapped in our efforts to measure the rate of conduction because oftentimes it is so hard to say just what points to take off from in the records. Sometimes there is a preliminary drop in the tracings from one or two places and pure rises in the others; and not infrequently it is hard to say from the graph just when the wave passed the recording points. Ordinarily, as we watch, we can see that the big sharp rise in the record comes just after the head of the column of intestinal contents has passed under the recording point. One thing seems certain, however, and that is that the rushes travel faster the farther they go down the bowel. When they first start, they may travel at the rate of from 2 to 4 cm. per second, but when they reach the lower ileum they will often cover from 7 to 25 cm. per second (average 9.9 cm.). Ordinarily in a rabbit, it takes about 50 seconds for a rush to travel from the pylorus to the ileocecal sphincter, a distance of some 300 cm. On measuring 65 such rushes from one end of the bowel to the other we found the mean rate to be 6.5 $\pm$ 1.8 cm. per second. The range was from 2.5 to 16.5 cm. There were two modes, however: one about 4.5 cm. and the other about 9.5 cm. Most of the data were grouped between 3.5 and 6.5 cm. Some of the faster rates may be due to errors in measurement but many seem to be due to the fact that the rush gathered momentum rapidly as it went down the bowel. Sometimes it can be seen that they are slowed up again for a few seconds before they are stopped. (See fig. 2 B in the paper on the Law of the Intestine.)

Two explanations immediately offer themselves for the increase in rate down the bowel: one, that the rush gathers momentum like a ball rolling down hill, and the other, that the conduction rate is a function of some graded peculiarity of the muscle from duodenum to ileum. The second

explanation can probably be ruled out because we find that those rushes which begin in the ileum and go a short distance travel as slowly as do those in the duodenum. When a rush travels rapidly over the ileum we may be pretty sure that it has come from the duodenum.

The next question arises: Why is there this acceleration? How could such a thing be produced? At first glance we can see three ways in which a wave might travel down the bowel. First, it might have to depend entirely on one strong push given to it at the start, much as a rifle bullet depends on one explosion, or as the ripples in a pond depend on one splash from a stone. In that case we would get retardation instead of acceleration. Second: The wave might travel like a spark along a train of gunpowder, in which case we would expect the rate always to be the same. Third: The wave might be produced by a force acting continuously, like gravity on a marble rolling down hill, in which case we would expect to see acceleration. One way in which such a force might act continuously would be through the mechanism of a gradient of forces such as we probably have in the bowel, hence it may be that the finding of this acceleration in the rate of travel of the rush waves down the bowel will later, when we understand it better, contribute something to the gradient theory of peristalsis. To be sure, it may contribute something also to the myenteric reflex theory because the myenteric reflex, when it acts must produce or steepen a gradient of forces.

The best explanation given so far for the progress of waves along muscular tubes seems to be that of Ralph Lillie (8) who believes that the action current produced by contraction at one spot stimulates the muscle next to it and so on. The wave spreads, then, somewhat like a spark in a gunpowder train, but that should travel at a constant rate in both directions and we know that in the bowel the wave spreads well only in one direction and then with a positive acceleration. When, as occasionally happens, a ripple runs orad a little distance from some loop of bowel which has just given rise to a rush caudad we find that the rate of progress orad is about 2 cm. per second, or much slower than the rate caudad. Similar observations were made during studies on the conduction of stimuli up and down the bowel (3). There we agree absolutely with Bayliss and Starling who found that the "excitatory effect. . . . rapidly diminishes in strength as it spreads up the intestine" and it can rarely be detected (in the dog) more than 3 cm. above the place stimulated. On the other hand, the inhibitory effect ran down the intestine rapidly—10 cm. per second—and they could trace it for two or three feet (9).

If we are to accept, then, the simile of a spark in a gunpowder fuse, we must imagine that the physical and chemical characteristics of the powder in that fuse are so graded that the spark can travel much more easily in one direction than in the other.

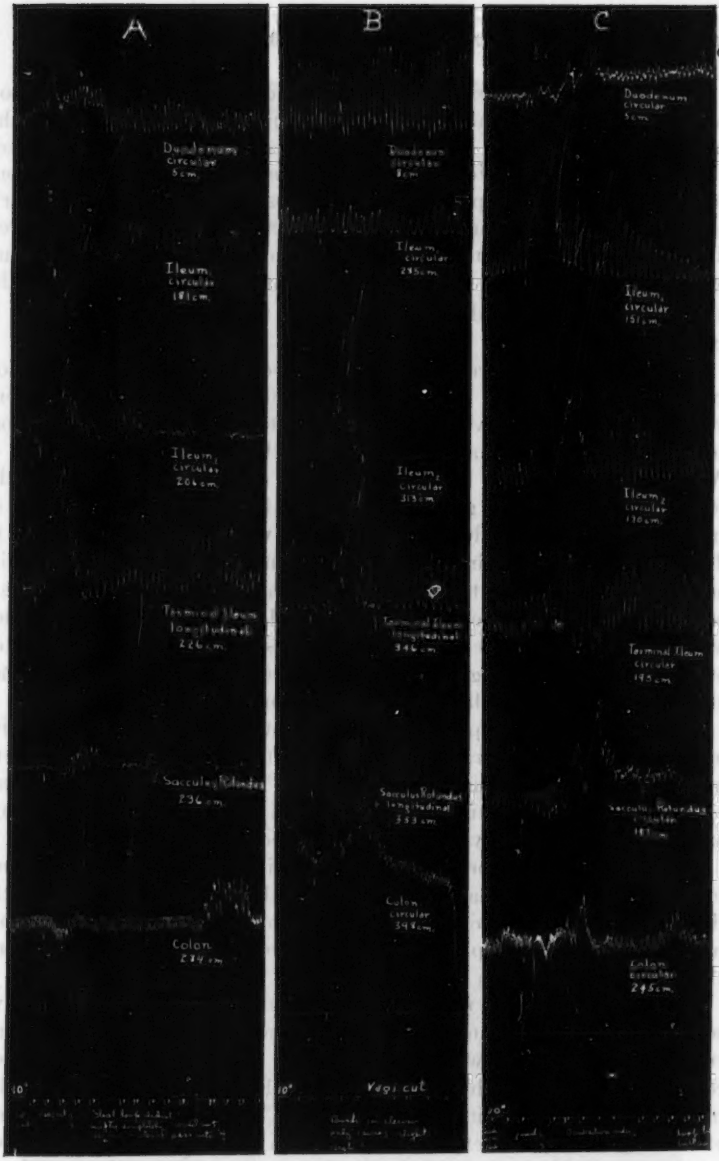


Fig. 3. A. Shows rise in duodenum synchronous with rush in lower ileum. Note that material was all left behind in 226 and only a ripple went on.

B. Shows drops in amplitude in duodenum as wave goes down the ileum.

C. Shows rise in duodenum shortly after a rush in the lower bowel. Note double peaks. Note also the sigh at the beginning of the disturbance. The big rises in the sacculus often stop the rushes.

It may be of interest to note here that Doctor Lillie finds no acceleration in the rate of conduction along his oxidized piano wires. In view of the fact that Tashiro thinks that there is a metabolic gradient along nerves it would be interesting to know if there is any acceleration in the conduction of the nerve impulse. There is a paragraph in Koenigsberger's *Life of Helmholtz* which suggests that that great genius, who first measured the rate of conduction in nerves, noticed local differences, but the literature seems quite silent on this point and Dr. Alexander Forbes tells me that the technic used so far could not be trusted to show acceleration even if it existed.

The gradient theory would lead us to expect faster rates of conduction in the animals with the steepest gradients (only the rhythmic gradient was measured), so we divided the animals into four groups with the most healthy and active animals in group 1 and the sickliest ones in group 4. We found then that the rhythmic gradients did average steeper and the conduction rate faster in groups 1 and 2 than in group 4, but there were so many disturbing individual variations that we feel that the evidence is, if anything, rather against the theory. One difficulty faces us again, however, and that is that we are not sure that the rhythmic gradient is a perfectly reliable index to the basic structural or metabolic gradient which we believe determines the direction of peristalsis. As one of us has pointed out before, the various gradients which have been demonstrated do not always run exactly parallel in the same animal (5).

It occurred to us that purged animals might have steeper gradients with more rushes and more rapid rates of conduction but in several experiments we did not find that to be the case. The purgation was produced by giving 10 cc. of tincture of aloes through a stomach tube. Perhaps another type of purgative would have served our purpose better.

In a few animals we produced some intestinal obstruction by pinching, bruising or cutting a place on the bowel. In some of these cases the rhythmic gradient was reversed on account of a rise in rate in the regions just above the injured place, and, as we would expect from the theory, the waves coming down were slowed.

Reverse ripples. In a number of records as in figure 3 A, B and C, we see that there is a rise in the tone of the duodenum very shortly after a rush wave has gone down over the ileum. More rarely there is a corresponding drop in the duodenal tone. Similarly, rush waves in the colon often inhibit the activity of the ileum. Unfortunately most of these effects appeared during experiments in which we had most of the recorders on the ileum and only one on the duodenum, so we cannot be sure that a ripple traveled up the bowel. At times the conduction rate was so fast, perhaps 100 cm. per second, that we must assume that the impulse traveled through the nerves either in the bowel wall or in the mesentery.

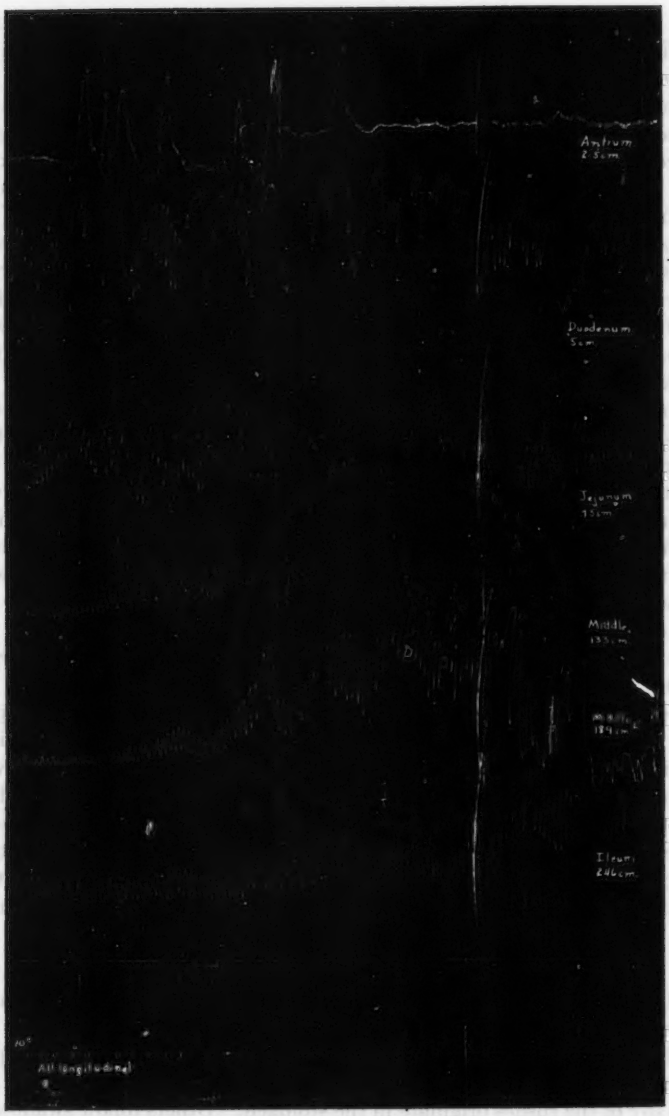


Fig. 4. Shows the increase in intestinal activity and the rise in tone which took place when considerable water seeped slowly into the bowel. Note the drop again when a rush came and partly emptied the loop 189 cm. from the pylorus.

In one cat we obtained some definite tracings of reverse rush waves running from the lower bowel to the stomach and terminating in vomiting. They will be published later.

Such reverse ripples and waves are of interest to the clinician because he has long noticed that people whose intestines are rumbling and overly active, especially at night, and who have a tendency to diarrhea, are likely next morning to have a coated tongue and a bad taste in the mouth, due apparently to regurgitation from the stomach and bowel (10).

Rushes in man. These can easily be seen with the roentgen ray screen, and so far as the senior writer can tell they behave exactly like those in the rabbit. The rate of travel seems also to be about the same. They can be studied also in men and women with fistulae into the small bowel. In one such case the senior writer noted that the giving of a little food or drink almost always started a rush just as it does in the rabbits.

Our animal studies throw light also on the behavior of the bowel in cases of diarrhea in men. When the bowel is unusually irritable and when the retarding mechanism in the terminal ileum and lower colon is not functioning well, it can easily be seen that the taking of a little food will generally start a rush which will gurgle down the bowel. This may produce the queer sinking feeling and then a call to defecation. We know also that if the stomach empties too fast, or if the food is cold or otherwise unusually stimulating, an abnormally large number of rushes may be started, and diarrhea may result. We know too that such stimuli are most effective in the morning after the night's rest when, as we have seen, the bowel seems to get on a hair trigger.

The Law of the Intestine and the mechanism which may perhaps produce and regulate the rush waves will be discussed in the following article. It will suffice here to call attention to the four different types of behavior of the bowel as the rushes pass. Sometimes there is preliminary drop in tone with or without the inhibition of one or two rhythmic contractions; sometimes there is a suspicion of inhibition about which we cannot be sure; sometimes the rise takes place sharply without preliminary inhibition or stimulation, and most frequently there is a rise of tone ahead of the rush.

SUMMARY

There are two main types of activity in the small intestine: rhythmic to and fro or segmenting movements, and peristaltic rushes.

The progress of rush waves down the bowel is now shown in graphic records obtained from six different regions at one time.

Many of the rushes originate in the stomach. Others follow swallowing movements, and many originate here and there in the bowel.

The rushes generally leave the pylorus just before a gastric wave arrives, and often before the stomach has had time to empty into the duodenum. A similar mechanism has been observed in man.

The emptying or filling of a loop anywhere may upset the equilibrium of the bowel and may cause one or more rushes in other loops.

There is a more or less systolic type of contraction which sometimes takes place in many parts of the bowel at one time.

Many rushes stop in the lower ileum, but some go on to the lower colon. They often bring about defecation.

Fortunately for us there are mechanisms which usually stop the rushes in the lower ileum.

The ileocecal sphincter serves not only to prevent regurgitation from the colon but also a too rapid emptying of the ileum.

The rushes often disturb the animal, causing sighs and movements of the feet.

The average rate of travel of the waves is 6.5 $\pm$ 1.8 cm. per second. They travel faster the farther they go down the bowel.

As would be expected from the gradient theory, the rate of travel above obstructions in the bowel was often slowed and at times the waves were thereby brought to a standstill.

Certain features in some of the records suggest the presence at times of reverse ripples or actually reverse waves. Reversed rushes ending in vomiting have been recorded in a cat.

BIBLIOGRAPHY

(1) Meltzer and Auer: This Journal, 1907, xx, 259.
(2) Alvarez: This Journal, 1915, xxxvii, 267.
(3) Alvarez and Mahoney: This Journal, 1923, lxiv, 376.
(4) Alvarez and Taylor: Journ. Pharm. Exper. Therap., 1917, x, 365.
(5) Alvarez: The mechanics of the digestive tract. New York, 1922.
(6) Cannon: This Journal, 1912, xxx, 114.
(7) Alvarez and Mahoney: This Journal, 1923, lxiv, 371.
(8) Lillie: Journ. Phys. Chem., 1920, xxiv, 165.
(9) Bayliss and Starling: Journ. Physiol., 1899, xxiv, 112.
(10) Alvarez: Journ. Amer. Med. Assoc., 1917, lxix, 2018.

PERISTALTIC RUSH AS DEPICTED IN THE ELECTRO-ENTEROGRAM

WALTER C. ALVAREZ AND LUCILLE J. MAHONEY

From the George Williams Hooper Foundation for Medical Research and the Department of Medicine, University of California Medical School, San Francisco

Received for publication March 29, 1924

While studying peristaltic rush we thought it might be helpful to study the electroenterograms obtained from several short segments of bowel as the big waves pass. We hoped that this technic might give us more exact information about the rate of travel of the waves and about descending inhibition. We obtained enough records to show that in some ways this technic is less suitable than the mechanical one because it brings with it some curious problems which we are not yet ready to solve. As we may not be able to return to the subject for some time we thought it well to publish here a few typical records together with a short discussion of them.

Technic. The animals were rabbits, anesthetized with urethane in the usual way. Their lower cords were pithed and their abdomens were opened under Locke's solution. The currents were led off with the help of small calomel electrodes made up with Locke's solution so that they would work in the bath in which the animal was placed. They were clamped gently against the bowel wall with little wooden clamps in such a way that a large part of the lumen was unobstructed and the rush waves could pass. The distance between electrodes on the bowel was 1 cm.

The tracings in figure 1 were obtained with three highly sensitive d'Arsonval galvanometers all recording on the same drum covered with bromide paper. Figure 2 was obtained with a Cambridge model of the string galvanometer with the string loosened so that 1 m. v. caused an excursion of 2 cm. The time record indicates 1-second intervals. As this record was rather faint, it was copied on tracing paper before it was photographed for reproduction here.

The records all read from left to right, and an upward deflection indicates negativity of the orad electrode. The signal marks the time when a rush was observed to pass one of the recording points.

Discussion. We note immediately that many of the rushes produce a surprisingly small disturbance as compared with what we have been accustomed to see in the mechanical records. Ordinarily the enterogram of a

rush looks like what we see in figure 1, B and C. The big swings of the mirror recorded in figure 1 D were observed only twice and in both instances they indicate a disturbance practically synchronous throughout the bowel or else an artefact. Another peculiar thing is that the rhythm seems often to be slowed when the wave passes, as depicted in figure 1 C. This may correspond to the big lasting contraction seen on the mechanical

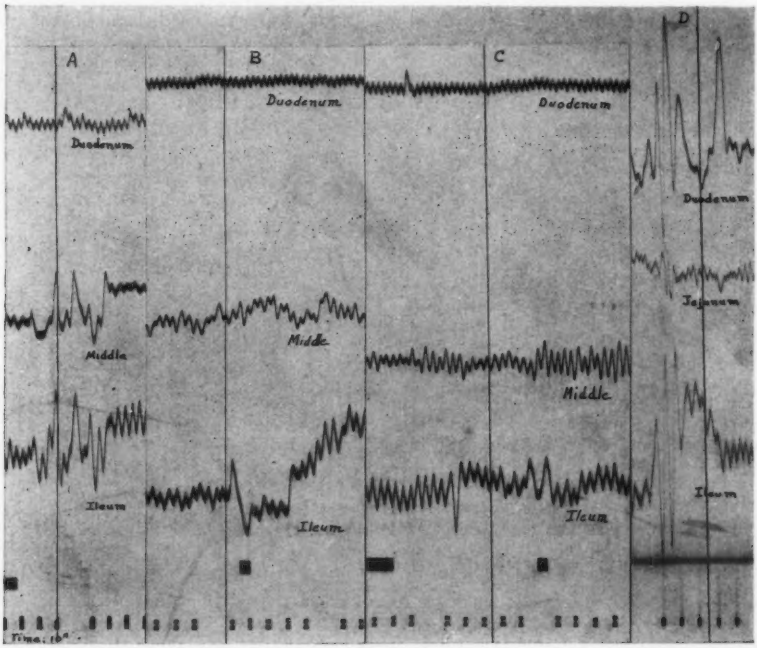


Fig. 1. Electroenterograms from rabbits. Details are given in the text. Vertical lines drawn to show synchronous points.

A. Signal marks injection of water in esophagus causing swallowing and a rush wave.

B. Signal marks passage of rush in the ileum.

C. First signal marks giving of water; second signal marks passage of rush in the ileum.

D. The big disturbance occurs at practically the same time all over the bowel and may easily be an artefact.

records, but the resemblance is slight. In casting about for an explanation of this peculiarity, it occurred to us that the rush wave might cause large voltage changes but the direction of the current might be reversed so rapidly that the d'Arsonval galvanometer, with its large inertia, could not record the disturbance properly. We therefore turned for a while to

the Einthoven instrument—long enough to see that that explanation is probably not the correct one. In figure 2 we see that a rush first increased somewhat the negativity of the orad electrode. There were no rapid voltage swings and the only resemblance between this electrical record and the mechanical ones which are to be found in the preceding paper on peristaltic rush comes in the period of relaxation after the rush. The large waves in the electrical record correspond to the rhythmic segmenting movements and the small ones superimposed are probably respiratory in origin.

It will be noted that there is no sign of a preliminary inhibition before the rush in figure 2. This point will be discussed in the next paper on the Myenteric Reflex.

Our results so far are in accord with those of other investigators who have found that the amplitude of the contraction of a muscle and the amount of voltage change in its action current do not always go hand in hand. In some of the records it looks actually as if the rush must have passed some of the segments without altering their rhythmic currents in any way. We observed also in a former article (1) that after paralyzing the bowel with adrenalin, good action currents could still be obtained. Similar observations have been made in the field of heart physiology.

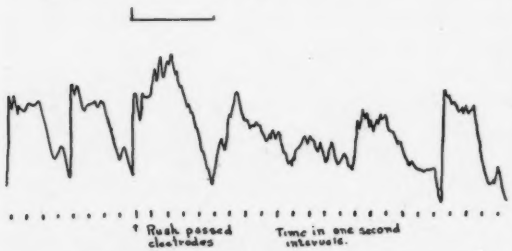


Fig. 2. Tracing from electroenterogram made with the string galvanometer. The signal above records the passing of the rush past the electrodes.

It is hoped that further work with the multiple electroenterograph can be reported upon during the next year.

SUMMARY

Electroenterograms have been secured of peristaltic rushes passing three different points on the bowel.

d'Arsonval and Einthoven galvanometers have been used.

Peculiar and quite marked differences have been observed between the electrical and mechanical records of rushes.

Signs of descending inhibition have seldom been obtained.

There seems to be no close correspondence between the amplitude of contraction in the bowel and the amount of voltage change in the action current.

BIBLIOGRAPHY

(1) ALVAREZ AND MAHONEY: This Journal, 1922, lix. 428.

BAYLISS AND STARLING'S LAW OF THE INTESTINE
OR
THE MYENTERIC REFLEX

WALTER C. ALVAREZ

From the George Williams Hooper Foundation for Medical Research, and the Department of Medicine, University of California Medical School, San Francisco

Received for publication March 29, 1924

In 1899 Bayliss and Starling published the first of a series of three classical and epoch making articles on the movements and innervation of the bowel (1), (2), (3). On page 110 of the first article we find the statement since quoted in every textbook, that "Excitation at any point of the gut excites contraction above, inhibition below. This is the law of the intestine." Cannon later proposed the term "myenteric reflex" for the phenomenon and showed that it could at times be elicited also in the stomach (4).

With this backing from three of the leading physiologists in the world, it is not surprising that this law or reflex almost immediately became one of the foundation stones of physiology about which there has never been any question or argument. Now, I must emphasize that my present purpose is not to deny that at times such a phenomenon can be observed, or to question the accuracy of the work of those who have gone before. I wish only to report some of the results of many years of work in this field: work which shows that the subject is not the closed chapter that all our textbook writers would lead us to believe. If they would only take the time to reread the articles written by the few men who originally studied this reflex, they would find there the frank admission that the phenomenon cannot always be demonstrated; that the animals have to be purged and the bowel denervated; that the reaction when obtained is often atypical or reversed; and that when present it is inadequate to explain many phases of persitalsis.

The main object of this paper, therefore, is to reopen the subject for further study and discussion. The question asked by Cannon in 1912 as to the causes which determine the appearance or non-appearance of the reflex when material is present in the intestine remains unanswered, and we do wrong in ignoring it so entirely.

Abstract of the literature. Bayliss and Starling did their first work on dogs which were anesthetized with morphine and the A.C.E. mixture.

The animals were given castor oil and then starved until the time of the experiment. The abdomen was opened under a bath of warm salt solution. In some animals the cord was destroyed; in others the splanchnics and vagi were cut, and in a few the abdominal ganglia were extirpated. Little difference could be found in the reactions of those intestines with complete destruction of the extrinsic nerves and those with section of the splanchnics alone or of both splanchnics and vagi.

In order to stimulate the bowel, they generally inserted recording balloons or cotton boluses covered with vaseline. Some records were made with an enterograph fastened to the outside of the bowel. My experience with balloons has left me much dissatisfied with them as there is so much trauma attendant up on their insertion, and the bowel often works so hard to get rid of them that it seems to me that the conditions are too far removed from normal. I am quite sure that these reactions of the bowel to the presence of a large foreign body are not exactly those which we see with normal peristalsis when there is only some semi-fluid material present. Similarly, Miss Mahoney and I hope soon to publish a series of records which show that the presence of an intestinal obstruction brings out types of persitalsis which we do not see under ordinary circumstances. The evidence obtained with the help of some form of enterograph attached lightly to the peritoneal surface of the bowel is undoubtedly much more trustworthy as an indication of what takes place in health.

Bayliss and Starling found little difficulty in demonstrating the "reflex" in dogs, and their tracings are quite convincing. We can see there the descending inhibition spreading out far enough ahead of the advancing balloon or bolus so that it could easily prepare the way for an advance. This inhibition was easily removed by fatigue which appeared after a few repetitions of the stimulus and caused the bolus to remain stationary in spite of the fact that waves of peristalsis ran over it. They found also that the orad and caudad effects do "not run absolutely parallel to one another, in that in some experiments the inhibitory effects from above, in others the augmentor effects from below may be better marked." Furthermore, "in many cases . . . . our results have not been quite so clear. Although stimulation above the balloon produced inhibition, stimulation below produced also inhibition or preliminary inhibition followed by contraction."

Their second paper deals with their attempts to show the law in the colons of dogs and rabbits. They remark upon the difficulty of showing descending inhibition in the rabbit, and note that when it is obtained, it is so evanescent that it is often hard to distinguish it from one of the normal rhythmic relaxations. When they came to work with cats they encountered even greater difficulties. These were somewhat obviated

when they purged the animals. They had difficulties also with the rabbit's small intestine. "An inhibition below the stimulated spot is not very apparent," and "it never extends down the intestine more than three or four centimeters." In the cat the contraction appeared over the center of the balloon, and not above it as in the dog. After purging the cats they concluded that the reactions were sufficiently like those seen in the dog, and they decided not to spend "more time in trying to ascertain the causes of the frequent absence of these reflexes."

It seems to me that so important a law should be easily demonstrable in all of the laboratory animals and then without the help of drugs and extreme denervation. The stress which they put on the importance of denervation has not been noticed by subsequent writers who have always deleted the first part of the law which really reads as follows: "One can indeed state this conclusion more generally, namely, *that if cerebrospinal reflexes be excluded* (italics mine) excitation at any point of the gut excites contraction above, inhibition below." It does seem as if this law should be demonstrable without such procedures because with the roentgen ray we can see innumerable rush waves going down the bowels of animals and men when the abdominal nerves have not been touched.

Magnus (5) demonstrated the reflex on excised strips of bowel stimulated by the application of salt crystals, but he admits that he could not obtain the expected reactions "with mathematical regularity." In some animals he got inhibition above and below. Langley and Magnus also had troubles while trying to show the law (6). They say: "it must be noticed that although inhibition below a point stimulated was so frequently obtained, it was in any one experiment, which lasted two or three hours, very inconstant; at one time the inhibition would be marked and at another absent, and the period during which one or the other state supervened was sometimes at the beginning of an experiment, some times an hour or two later." Degenerative section of the great majority of the superior mesenteric nerves did not abolish the reactions, showing that their mechanism must be located in the bowel wall. Elliott and Barclay Smith noticed a typical reaction in the colon of a cat starved three days (7).

Cannon realized fully that the myenteric reflex is not always demonstrable and that it does not have anything to do with many of the rhythmic activities of the tract. As he says: "What causes (it) to appear or not when material is present is as yet undetermined. . . . . It does not govern the rhythmic contractions of the small intestine, the rhythmic peristalsis and antiperistalsis of the colon, and probably not the rhythmic waves of the stomach" (4).

Wheelon and Thomas (8) recently had occasion, while studying the activities of the stomach and duodenum, to look for signs of the reflex but they were unable to demonstrate it.

In 1913 I obtained the first records of peristaltic rushes passing six or seven recorders attached to the bowels of rabbits, and I was surprised to find that ordinarily the bowel not only did not relax in front of the advancing wave but it often became more active and served to stop the rush, especially in the lower ileum (9). The beneficial effects of such a mechanism were immediately obvious, however, because without it, if the myenteric reflex were always active, our bowels would soon be empty and we would die of starvation.

In 1918 Miss Starkweather and I studied the graphic records of the contractions resulting from over two thousand mechanical, electrical and chemical stimuli applied to the peritoneal surface of the intestine (mainly of rabbits), and found that the usual response in all but some twenty experiments was contraction above and below (10). The fact that most of the few responses which were in accord with the law were obtained by distending the bowel with balloons made us feel that possibly the law should be restated, in that it may be applicable in the case of distending foreign bodies but not in that of external stimuli, such as pinches and faradic shocks.

One objection to the work done by Miss Starkweather and me might be that we generally recorded the movements of the longitudinal muscle, but neither we nor Bayliss and Starling found much difference between the reactions of the two coats. Another objection which has been raised by some is that the intestines were not denervated. This seems hardly valid because our rabbits were pithed just as Bayliss and Starling's were, and besides, we obtained the same reactions with segments of bowel removed from the body and placed in oxygenated Locke's solution.

Results of the present inquiry. During the last two years Miss Mahoney and I have been getting good records from six or seven parts of the bowel at one time. The technic has been described in the preceding article on peristaltic rush (11). Briefly, the animals have generally been rabbits; they have been anesthetized with urethane (2 grams per kilo by mouth); their lower cords have been pithed; their abdomens opened under a bath of Locke's solution, and short segments of their bowels connected by means of threads to light heart levers. Ordinarily the longitudinal muscle has been used, but we often have recorded the contractions of the circular muscle also. Little if any difference can be seen between the two sets of records. The vagi were cut below the diaphragm in several animals. The only difference observed was a lessening of the number of rushes down the bowel.

Some three hundred feet of records have been obtained with this technic and a number of specimen tracings are shown in this and in the preceding paper. We have now the opportunity of studying the movements of the bowel as they should be studied—while they are engaged in their

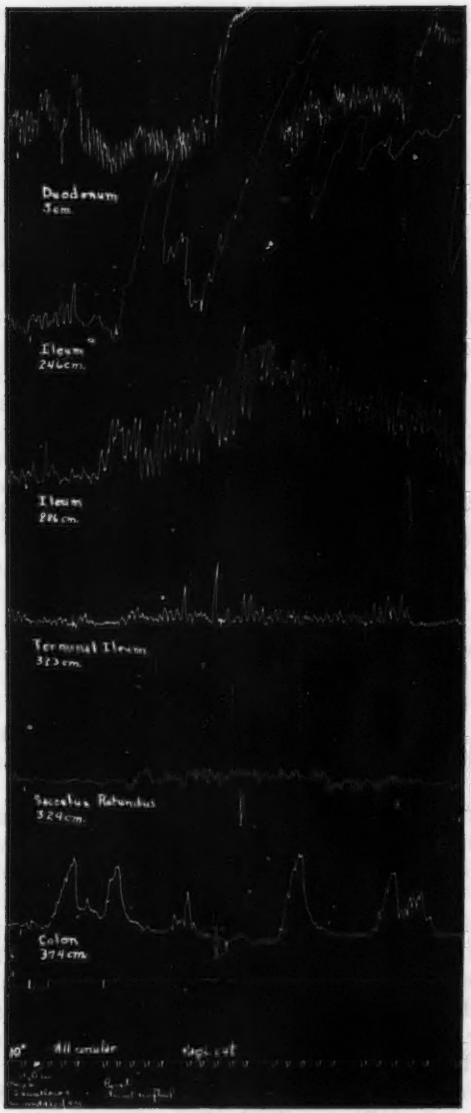


Fig. 1. Shows stoppage of a rush in the lower bowel due to powerful contractions of the ileum. No material could be seen to pass the ileum 286 cm. from the pylorus, but apparently some ripples went on as far as the terminal ileum. Possibly some material seeped by to help increase the activity of the terminal ileum and sacculus.

normal processes of digestion and not while they are struggling with foreign bodies or suffering from the effects of unusual stimuli and trauma.

The big rises in the records coincide with the passage of the column of intestinal contents under the recorder. All records read from left to right. Contractions of the bowel are recorded as upstrokes, and the time record indicates ten second intervals. Small marks on the records indicate synchronous points.

As we look over these records, depicting the activities of the bowel during peristaltic rushes, we see that a descending inhibition is often absent. In fact, the most frequent finding is a gradual rise lasting for from ten to twenty seconds before the crest of the wave arrives. This occurred in 38 per cent of 239 records. No inhibition could be detected in another 27 per cent; a slight, questionable inhibition was found in 20 per cent; and a definite drop in the level of the record or, more often, an inhibition of one or two rhythmic contractions was found in 15 per cent.

In rough figures, then, some inhibition is found in at most a third of the cases while in the other two-thirds there is either no change or else a preliminary rise—against the law. Very seldom has there been any great drop in tone as in figure 2 A and rarely has it looked as if the inhibition were lasting enough or definite enough to help much in smoothing the way for the rush. A glance at the records will show that the absence of deep drops in tone is not due to any inability on the part of the bowel to have them; but, unfortunately for one who is trying to demonstrate the law, they almost always come just *after* the rushes have passed. Such relaxation after the rush is due apparently to the quieting down of the bowel after it has emptied itself of its contents. The drop in tone probably makes it easier for material to come down from regions of higher tension orad, and there may also be some slipping back of material from loops caudad.

Another difficulty is that we have seldom been able to say definitely that a drop in the level of the record means inhibition because as we watch the waves passing the recorders, it looks as if the distention brought about by the advancing column of fluid must often be the responsible agent. It must be remembered also that there are no fixed points on the bowel, so that the contraction of the longitudunal muscle fibers at one point must exert a pull upon, and must stretch the more quiescent fibers immediately above and below. (See fig. 3 A.) Similarly, a contraction of the circular fibers in one segment, if it forces material into the loops just above and below, may dilate those loops and may thus lengthen the muscle rings recording on the kymograph. (See fig. 3 B.) Under the circumstances, it is really remarkable that most of our intestinal records show no sign of what might be interpreted as due either to stretching or to inhibition. The stomach records are the ones which are most deformed

in this way; so much so sometimes that they are almost uninterpretable. The comparative freedom of the intestinal records from signs of distention seems to be due to the fact that the bowel is generally beginning to contract as the material arrives, and the two opposing forces neutralize each other for a few seconds until the contraction wins out.

Evidence from the electroenterograms. We tried to differentiate between inhibition and passive stretching by recording the electrical currents which are produced during the contraction of the muscle, but unfortunately, some recent research work (12) shows that the electrogram cannot be counted upon to help us in this difficulty. Actually, however, it has been of assistance because most of our electric records show no sign either of inhibition or stretching before the rush. See illustrations in the preceding paper.

Variability of the reaction. As will be seen from the first paper, on peristaltic rush, one of the great difficulties in measuring the rate of the rushes down the bowel arises from the fact that loops a few centimeters apart respond so differently that comparable points of take-off are lacking. Thus, at one point there may be a gradual rise in the tone of the bowel for ten or twenty seconds before the crest of the wave arrives; 50 cm. farther caudad the bowel may show slight "inhibition" for perhaps three seconds before a sudden and powerful contraction takes place; the segment next below may show such a gradual rise and fall of tone extending over sixty seconds or more that we cannot say exactly when the rush passed; and the next segment may show a sharp, clear-cut rise without any preliminary change one way or the other. Five minutes later another rush may sweep over the bowel with a different type of response from the various segments, showing that their varying behavior must depend upon momentary changes of some kind.

We should not be surprised at this, however, because smooth muscle is notoriously unreliable in its reactions to stimuli. The literature on the effects of vagus and splanchnic stimulation on the bowel is a maze of contradictions because the muscle responds with so many different combinations of relaxation and contraction, and everything depends apparently on the state of the organ when the stimulus arrives: i.e., whether it is fatigued or fresh, contracting or relaxing, etc. It is this unreliability of smooth muscle that probably accounts for the great variety of reactions which Bayliss and Starling, Magnus, Langley, Cannon and I have obtained.

I was interested to see whether or not a sudden filling of a loop, with the resultant great increase in activity (corresponding somewhat to the experiments with balloons) would have any characteristic effect on loops just above or below, but apparently there is none. (See fig. 4 A, B and C.) Often there is a drop in tone and activity in the loops above, but

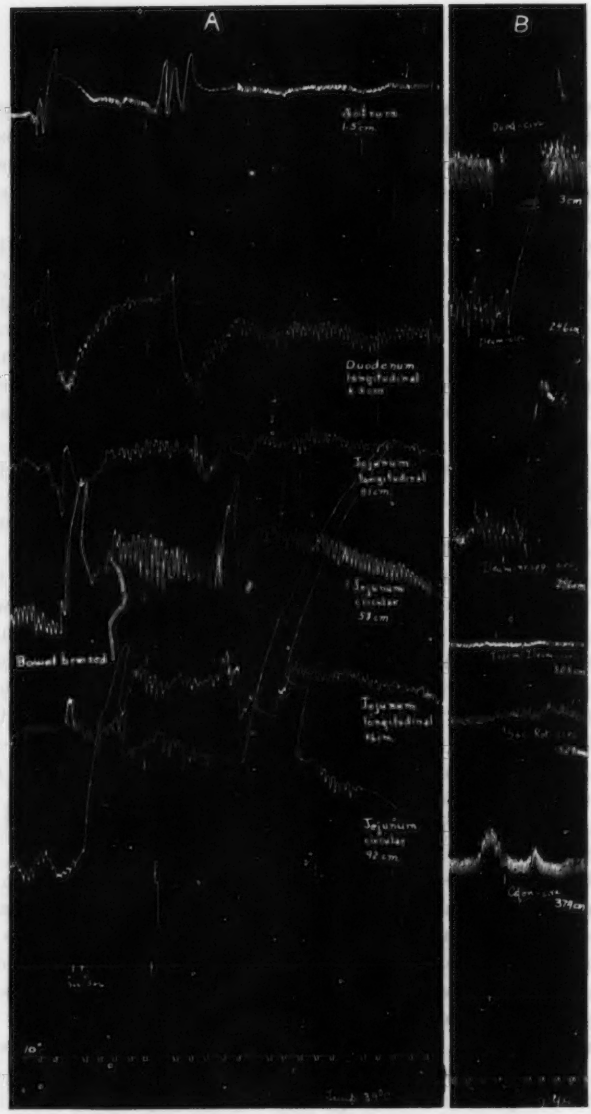


Fig. 2. A. Shows a rush passing over a bruised place and also through a loop of jejunum which was tonically contracted. Note big drops in the tone of the duodenum after rushes. Note influence of the stomach in starting rushes. Note few descending inhibitions in the jejunum.

B. Shows a rush traveling slowly, 2.6 cm. per second, just before it was stopped in the lower ileum. Note the increase in the tone and activity of the sacculus and colon, beginning probably about the time the rush left the upper bowel. Note also the rise in the duodenum beginning 5 seconds *after* the rush reached the lower ileum.

this is probably due, not to inhibition, but to the fact that they lost some of the material which was keeping them active.

Discussion. Any one who has read thus far will probably be impressed by the fact, noted also by all previous workers in this field, that the myenteric reflex is unreliable and often absent. The question then arises: What significance has it in the mechanics of the digestive tract? Can the bowel get along without it, and if so, what makes the waves go down the bowel? How do they keep going when the reflex is reversed?

Bayliss and Starling were apparently so thoroughly convinced of the supreme importance of the law that they were willing to say that when either the contraction above or the inhibition below is absent, "the onward movement of the bolus becomes impossible." It is interesting to note, however, that in one of their experiments, when the reflex was abolished through fatigue and the bolus would not budge, a peristaltic wave still went over it and on down the bowel (1, p. 108). That is just the sort of thing which we have observed repeatedly: i.e., a dissociation between the wave of contraction in the muscle layer and the movement of material through the lumen of the bowel. Something of the kind was probably in Cannon's mind also when he said that the "reflex and the progression of the reflex along the intestine are not the same phenomenon" (*Mechanical Factors*, p. 183); and it is a commonplace observation for the roentgenologist, who must often wonder why a few of the waves running over the stomach grip the contents and pass them onward, while others, apparently just as powerful, have no effect. It makes one think of some sort of a clutch which is let in to connect the intestinal engine with the work it has to do.

Rushes can jump gaps or obstructed places in the bowel. In figure 3 B it will be seen that a rush can go on down the bowel after it has had to leave its burden of intestinal contents behind. This was brought about by tying the bowel across with a bit of tape. It is probable that as the rush wave in the wall went on it picked up some new material on the other side of the obstruction. In figure 3 A in the preceding paper on peristaltic rush, and in figures 1 and 5 C of this paper there are signs of ripples which went onward while the intestinal contents remained behind.

Similarly, we can stop the progress of the wave in the wall without stopping the onward rush of the intestinal contents. In order to produce this we either bruised a ring of bowel by pinching it for a moment with a clamp, or else we cut the gut across and immediately restored its continuity with a crude end to end anastomosis. Sometimes, with the traumatized bowel, the reversal of the gradient (?) was sufficient so that both the rush waves and the contents were stopped and turned back above the pinched place. More often, however, the wave faded out, and so far as we could see, disappeared; but the column of intestinal

contents was long enough so that it was forced through the damaged area and into the normal bowel beyond. There the presence of material resulted in contraction of the muscle, and a new wave carried the material onward without any interruption. (See fig. 3 C.)

The question next arises: May not such coördination be maintained with the help of the nerves in the mesentery, much as Meltzer found it to be maintained in the esophagus by the vagi? In order to answer that we did a few experiments in which we simply cut the bowel across and in those we could see no sign of any disturbance jumping the gap. Long distance coördinations through the mesenteric nerves may have remained, but we were not looking for them so much as for disturbances in the loops immediately below the gap.

As some work on ctenophores and spermatozoa (13, p. 33) has shown that waves can jump gaps with the help of the action currents, it was thought best to rule out that possibility by cutting the bowel in two, and then tying the ends over a glass tube 5 mm. in inside diameter and 3.5 cm. long. The two ends of intestine were thus separated by 1.5 cm. of glass. To be sure, they were immersed in Locke's solution but it does not seem likely that the feeble action currents could jump such a gap with enough strength left to stimulate the muscle on the other side. Moreover, it seemed clear as we watched the rush waves pass, that the contraction in the wall stopped 3 or 4 cm. above the gap and the new one appeared 2 to 3 cm. below. Figures 5 A, B and C show graphic records of these experiments.

In designing these experiments I had in mind the work of Friedlander (14) who cut an earthworm in two and tied the pieces together with a bit of thread. The hind end crawled on in coördination with the front end simply because the pull on the skin supplied a sufficient stimulus for the underlying muscles. Similarly, in the bowel we may find later that some reactions which now seem complicated are really simple and local, and due to stretching or to the presence of chemical irritants within the tube. Incidentally, there are many things which suggest that Cannon is right in assuming that the character of the intestinal contents has much to do with determining the nature of the local muscular response.

Fig. 3. A. Shows powerful contractions in the duodenum due to an obstruction. The apparent inhibitions in the loop above were due plainly to a stretching produced by the marked shortening of the muscle in the active loop. Similar depressions were produced in the record from the loop just below.

B. To show how a rush wave can go on down the bowel after it has left its freight of intestinal contents above a place where the bowel has been tied tightly. Note also the drop in the fourth record due to distention of the recording loop.

C. To show a rush traversing a section of bowel which has just been cut in two and then sewed together again, end for end. The big rises just above and below the cut place were practically synchronous. Note the systolic type of contraction in the bowel following the rush.

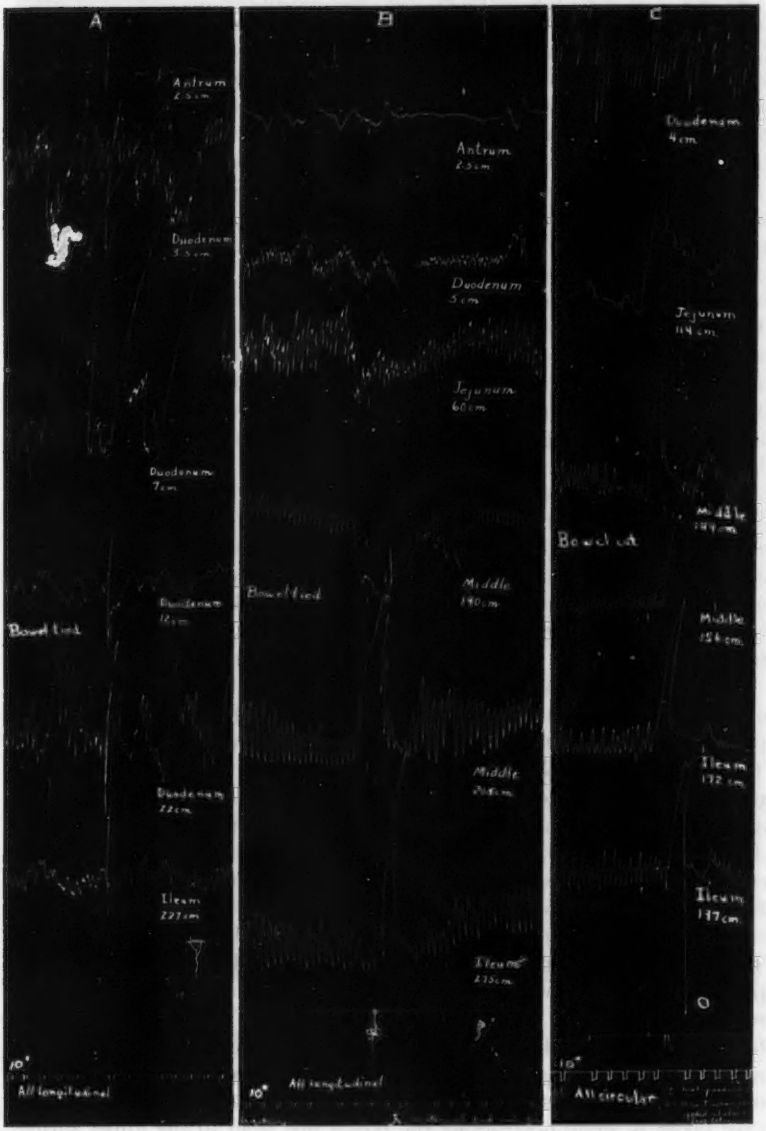

A
Antrum
2.5 cm
Duodenum
3.5 cm
Duodenum
7 cm
Duodenum
12 cm
Bowel tied
Duodenum
22 cm
Ileum
227 cm
10°
All longitudinal
B
Antrum
2.5 cm
Duodenum
5 cm
Jejunum
60 cm
Bowel tied
Middle
190 cm
Middle
Ileum
275 cm
10°
All longitudinal
C
Duodenum
4 cm
Jejunum
114 cm
Bowel cut
Middle
154 cm
Ileum
172 cm
Ileum
147 cm
10°
All circular

Fig. 3

We see then, first, that downward peristalsis can take place without the help of the myenteric reflex: in fact a glance at figure 2 A will show that a rush wave can go on sometimes even when the bowel ahead of it is powerfully contracted; and secondly, we see that any discussion of the subject is inadequate unless it deals separately with at least two, more or less related, phenomena, i.e., the wave traveling down the muscular coat of the bowel and the progress of material within its lumen.

The wave in the wall travels probably much as does the impulse in the heart, with the action current spreading out ahead and stimulating the muscle in some such way as R. S. Lillie has described (15). The direction of spread is determined probably, as in the heart, by a gradient of rhythmicity and metabolism; and so far as this muscular ripple is concerned, there is probably no more need for a descending inhibition than there is in the heart. At times this ripple becomes deeper and changes its characteristics in some way so that it grips the contents of the tube and forces them onward. Under those circumstances there sometimes is a wave of inhibition spreading out ahead of the advancing column—just why, we do not know.

To my mind the best evidence of the presence of some such relaxation is found in the fact observed by Bayliss and Starling on animals and by me on man that a balloon connected to a manometer and allowed to run down the bowel does not show much sign of being pressed upon. It seems to slip along without any great effort on the part of the gut. This lack of effort and conflict perhaps also accounts for the fact that the electroenterogram shows so little disturbance as the rush waves pass.

Difference between transport of solids and fluids. Any discussion of the myenteric reflex must take into account also the difference between the problems involved in the passage of solids and fluids through the intestine. These problems are quite comparable apparently to those involved in passing rocks and water through a pipe line. The water will go anywhere, up hill and down, if enough pressure is put on it, but the rocks will go only down hill. Similarly, in the bowel, liquids will seep up and down for considerable distances without the help of any definite rush waves. (See fig. 4 in the paper on peristaltic rush and figs. 4 A and B here.) Water can even be pumped into an animal's rectum until it flows out of its mouth. This difference between the behavior of solids and liquids can be seen perhaps best in dogs which have had a segment of small intestine cut out, reversed end for end, and anastomosed again. If the animals are then fed very carefully with foods which are soon reduced by the stomach to a liquid state, all goes well, but if they get a chance to eat bits of wood or bone or straw, they soon die, and every scrap of such solid material ever taken is found obstructing the bowel just orad to the upper suture line (13, p. 38). There can be little doubt, then, but that the reversed

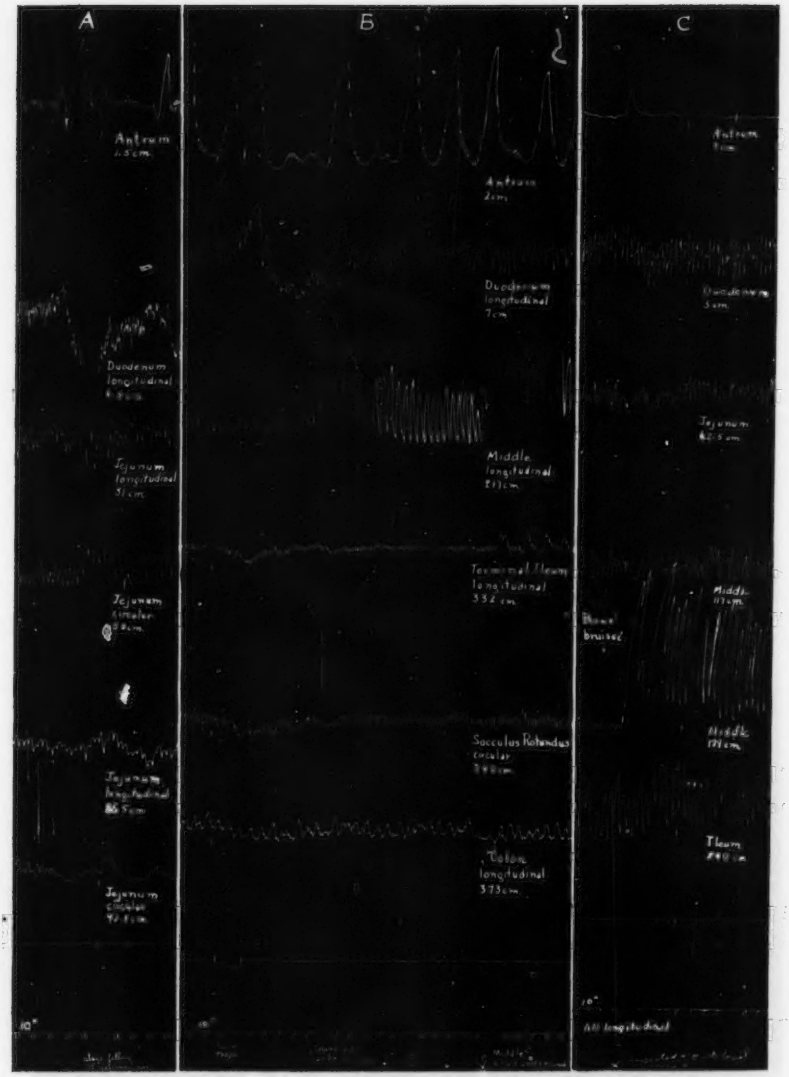


Fig. 4. A. Shows slow emptying of the duodenum into the first loop of jejunum. Note slight increase of tone in the bowel below and the drop in tone of the empty duodenum.

B. Shows again a slow emptying of the duodenum into the loops just above the middle recorder. Note: no descending inhibition either then or later when the material seeped under that recorder. Note the immediate increase in the activity of the bowel 115 cm. caudad.

C. Shows the effect of injecting some water with a hypodermic needle into the bowel just above the loop 117 cm. from the stomach. Note absence of descending inhibition.

segment has maintained its gradient, and this has made an "up hill" stretch in the bowel like a siphon in a pipe line. Fluids can be forced over it but not solids. We know also from the work of Muller and his associates (16) that dogs will live comfortably after all the muscle has been stripped from long stretches of bowel—if all rough food is kept from them. Returning to the simile of the pipe line, this must mean that fluids can be pumped through long stretches where there not only is no gradient but no motive power.

Why rushes can pass an obstruction. The experiments just described will perhaps help us to understand why the peristaltic rushes can whisk on down the bowel when the records show so clearly that instead of relaxation there is a definite contraction of the bowel ahead of them. First we must note that the advancing column of intestinal contents is often several centimeters long. With the motive power at the orad end, it may be likened somewhat to a train of cars with the locomotive pushing. Next, we must look carefully at the records for a moment, and must do a little comparing of the width of the big rush contractions with the time record. This will show us that these contractions last from ten to twenty-five seconds. Turning back to the paper on peristaltic rushes, we will see that their rate of travel varies between 2.5 and 16.5 cm. per second. That means that by the time the contracted segment relaxes, the head of the rush wave must be from 25 to 400 cm. farther caudad and well on its way down the bowel. Under these circumstances it can easily be seen that once a wave is started in one direction it must continue in that direction so long as the bowel is irritable enough to respond locally to the presence of the column of fluid, and so long as the resistance ahead does not become too great.

The next question is, how does it get started in the caudad direction? Material put into the middle of the bowel should, with this mechanism alone, go equally well in both directions. Actually it does tend to do that, but probably on account of the presence of the gradient in the wall, it is hard for the muscular wave to go orad and it soon fades out. The caudad wave, running down the gradient, gathers momentum and runs faster the farther it goes. Matters are simpler probably when the rush starts as it usually does from the pylorus which can close behind it and can prevent regurgitation. To use a Hibernianism, food goes down the bowel more easily because it is put in at the upper end!

Theoretically, then, if we were to put irritating material into the terminal ileum and if we could get the ileocecal sphincter to close behind it, we might produce a reverse rush. Actually I have been able to do this in two cats but only after raising the irritability of the colon for some time by causing it to contract powerfully on some soapy water. The rectum, naturally, had to be tied off. Apparently the lower end of the

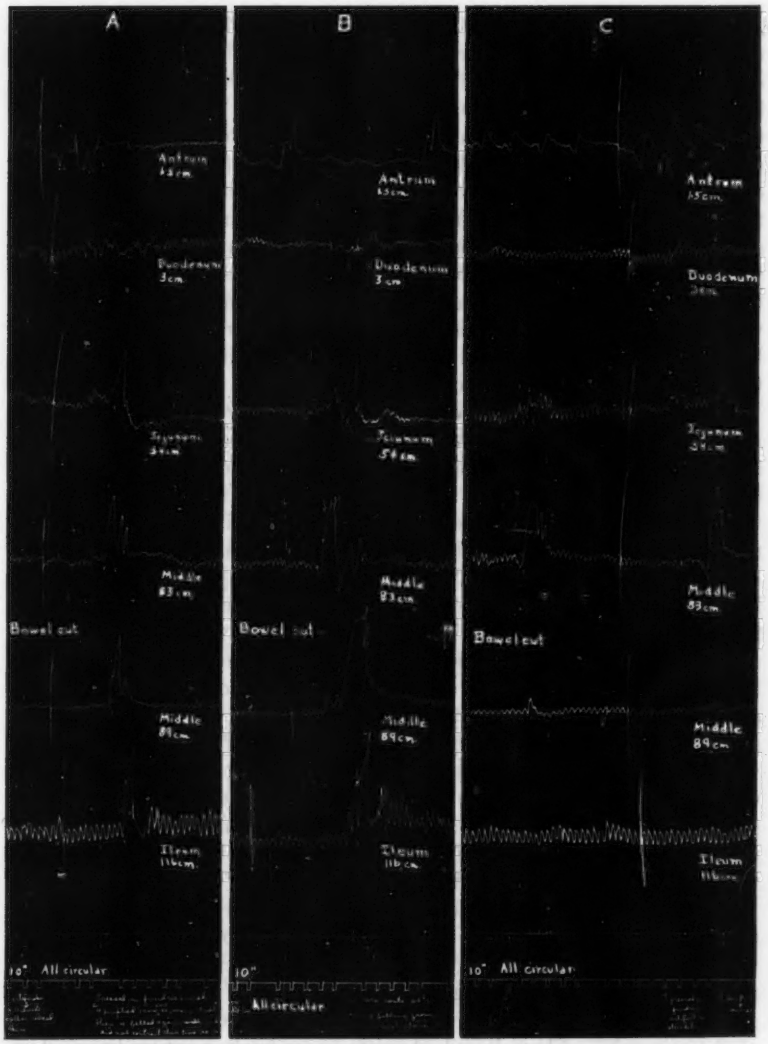


Fig. 5. The bowel was cut across and the ends tied over a piece of glass tubing 3.5 cm. long and 5 mm. in inside diameter.

A. A rush passed. The bowel below the tube contracted 2 seconds before the rush reached the upper end, showing again that it was a new wave, arising in response to the arrival of material from above.

B. Note some advancing contraction even below the cut.

C. Rushes carrying material were stopped above the cut but a ripple went on. Possibly some seepage occurred through the tube into the bowel beyond.

gradient had first to be raised a bit in this way. The wave, which ran up as far as the duodenum, seemed very plainly to be due to the intense irritation of the bowel as the soapy colonic contents reached successive loops. This material had to move orad because the bowel remained contracted for so long a time behind it that there was no chance for any regurgitation. Recently, in a cat with a poor rhythmic gradient, we have obtained records of spontaneous reversed rushes running from the ileum to the stomach and producing vomiting. It is interesting that these show the same mixtures of preliminary inhibition and contraction which we have seen with the descending waves. If the rush waves can run up the bowel in the same way that they run down, a number of investigators will have to give up their ideas of short nerve tracts in one direction and long tracts in the other, and other similar anatomic devices to explain the myenteric reflex and the downward direction of the normal waves.

The gradient idea can give us still another way of looking at those rushes which advance through contracted and resisting stretches of bowel. We need only to imagine a pipe line laid down a mountain side and then up a little way over a hill at the foot of the mountain. If some water were to rush down the pipeline it could easily be carried by its momentum over the top of the foothill, granting that that hill were not too high. If it were too high, some or all of the water would not get over but would fall back into the valley, and that seems to be exactly what happens when a rush wave is stopped somewhere in the bowel. The resistance ahead of it becomes too great, and some or all of the material being transported is stopped or thrown back. At other times the wave seems to stop largely because the bowel (usually lower ileum) is insensitive and does not respond sufficiently to the presence of the material.

We see then that the bowel can easily handle the transport of fluids without the help of the myenteric reflex and even with the handicap of a reversed reflex. For the handling of solids, however, it seems pretty clear that the gradient must be just so; and it is probable that the myenteric reflex must also be working right.

Clinical applications. Incidentally, we have here some observations of great clinical value because if the tract must be working just so in order to transport solids, it is plain that we must be most careful to use a perfectly smooth diet during digestive upsets or after operations when the gradient is probably poor or reversed; also when there are irritative lesions of the stomach and bowel, and when there are healing suture lines. Unfortunately, the craze at present is to stuff even the weakest invalids with spinach, salads, fruits, bran and other coarse foods which have a large amount of indigestible residue. Actually, in practice it works much better in many cases to use a "smooth" diet which is easily reducible in the stomach to a pap which can then be made to flow quite easily "up hill" and past obstructions.

The rather limited application of the law may account for the fact that it has been of so little use to the clinician in his attempts to understand the deranged action of the bowel in disease. The law acts generally over short stretches of bowel, but what the clinician wants is an explanation for correlated phenomena which appear often times at the two ends of the digestive tract. Many of these things can be explained only with the help of the gradient idea.

I might mention the fact that there is some question whether or not the myenteric reflex is a real reflex with some kind of nervous arc in Auerbach's plexus. A number of anatomists have failed to demonstrate any such arc, and Gaskell (17) has stated his belief that at most it can only be an axon reflex. There is also my work with strychnin which failed to show any sign of synapses in the excised bowel (10). As would be expected, when we cut the bowel across and sewed it together again, the recorder attached 2 cm. below the cut usually showed no sign of either preliminary relaxation or contraction as the rush waves went by. Strange to say, on one occasion (see fig. 5 B) there was a preliminary contraction.

In connection with this question of the mechanism underlying the myenteric reflex there are a few suggestive things which may be mentioned. In the first place, examples of what might be called contraction above and inhibition below have been observed in other hollow tubes and in lower animals which have nervous systems somewhat different from the one found in the bowel. For instance, v. Uexkull (18) has described what he calls the noose reflex in the leech. If the animal is tied lightly with a woolen thread about its middle, a contraction appears orad to the noose and relaxation caudad, and the thread is thus shifted backwards until it drops off. Fry (19) found also that in the heart of one of the squids where the beat is peristaltic in character, stimulation of the visceral nerve produced contraction of the auricle and relaxation of the ventricle. The threshold for the two effects was the same, but with very weak shocks the effects were reversed. It may be that the study of some of these myenteric-reflex-like reactions would throw light on the mechanism with which we have to deal in the bowel.

It is interesting also that many small organisms or their appendages respond perhaps with inhibition to a weak stimulus and with contraction to a stronger one (20), and Spadolini (21) has observed similar things in the intestine. As conduction in the bowel is better caudad than orad we might conceive of a difference in the behavior of the muscle above and below an irritated place as being due to a difference in the strength of the transmitted stimulus. So far our experiments with pinches and electrical stimuli offer no support to this view because the response is almost always a contraction above and below.

A suggestion may be offered here in explanation of the fact that the most powerful contraction comes at the orad end of a column of fluid in the bowel. It has been shown pretty definitely by v. Uexkull (22) that smooth muscle in connection with a nerve net responds first at the point where the tension is greatest. Now if the tone of the muscle in the tube were everywhere the same: that is, if there were no gradient, and if the bolus or column of fluid were perfectly cylindrical, the distending force would be equal along that column and there would be no more tendency for the muscle to contract in one place than in another. There are two ways, however, in which the contraction might be thrown to one end or the other. The bolus might be somewhat conical like a blackboard crayon, in which case the muscle would be stretched most at the end with the greater diameter; or the primary tension or tone of the muscle might be graded as Trendelenburg (23) and I believe it is, in which case a purely cylindrical bolus would stimulate most at the end of the loop with the more tonic muscle.

SUMMARY

According to Bayliss and Starling: "If cerebral reflexes be excluded, excitation at any point of the gut excites contraction above, inhibition below. This is the Law of the Intestine."

The few men who have written on this subject all report that the demonstration of this reflex is not easy. It is often absent, atypical or reversed. Cannon recognized the limited applicability of the reflex and did not regard the subject as the closed chapter which it has since become.

In the rabbit, stimuli applied to the outer coat of the bowel practically always produce contraction above and below. This suggests the need for restating the law. It may perhaps be applicable only in cases in which the bowel is distended or stimulated from within.

The subject is now being investigated with a new technic. Instead of traumatizing the bowel with balloons and other foreign bodies the normal rush waves are studied as they pass several points in the bowel.

What might be interpreted as signs of descending inhibition were found in about a third (15 per cent definite; 20 per cent questionable) of 239 records. A contraction ahead of the wave (against the law) was found in 38 per cent, and in another 27 per cent there was certainly no inhibition. What looks like inhibition on the records may easily be the result of stretching of the recording segments by intestinal contents or by the pull from neighboring loops.

Rarely were there signs of such drops in tone as might be of definite help in forwarding the waves.

A marked drop in tone often *followed* the passage of a rush.

The reactions of any one loop to a series of rushes were very variable. This irregular behavior is typical of smooth muscle generally.

It appears now that in any discussion of the myenteric reflex we must distinguish clearly between the muscular wave in the wall and the movement of material in the lumen because not only can the wave at times go on and leave the material behind, but under certain conditions, the wave may stop at some barrier while the material goes on to start a new wave on the other side.

The wave in the muscular wall probably follows a rhythmic and metabolic gradient, just as the contraction does in the heart. It probably has no need for any descending inhibition.

We do not know exactly what change comes over this wave when from time to time it suddenly deepens and forces material down the bowel. Neither do we know why descending inhibition sometimes then appears.

The resistance which we usually find springing up ahead of the advancing wave must serve a useful purpose in keeping the bowel from emptying too rapidly.

Peristaltic rushes can easily jump complete breaks in the continuity of the bowel—bridged by a short piece of glass tubing.

Many experiments show that conditions in the bowel must be just so if solids are to be moved. Liquids can easily be forced anywhere up gradients and past obstructions.

The waves travel in one direction largely because the contraction moving behind them is so lasting that there is no chance for regurgitation. By putting irritant material into the bowel above the ileocecal valve in cats, reverse waves can be obtained, running the length of the small bowel. Spontaneous reverse rushes in the small bowel have been recorded in the cat.

Many of the manifestations of peristalsis can best be understood with the help of a pipe line simile described in the text.

The studies here reported show how important it is to use a smooth residue-less diet in many gastro-intestinal diseases.

Suggestions are offered to explain some of the workings of the myenteric reflex. It is a question whether it is actually a reflex.

It would seem clear that we cannot go on saying simply that waves go down the bowel on account of the law, or the reflex. In the rabbit the reflex can be demonstrated in perhaps a third of the rushes and the waves go on just as well when it is reversed.

The subject is still open for study and discussion.

BIBLIOGRAPHY

(1) BAYLISS AND STARLING: Journ. Physiol., 1899, xxiv, 99.
(2) BAYLISS AND STARLING: Journ. Physiol., 1900, xxvi, 107.
(3) BAYLISS AND STARLING: Journ. Physiol., 1901, xxvi, 125.
(4) CANNON: This Journal, 1912, xxx, 114; The mechanical factors of digestion New York, 1911, p. 195.
(5) MAGNUS: Pflügers Arch., 1904, cii, 132.
(6) LANGLEY AND MAGNUS: Journ. Physiol. 1905, xxxiii, 34.
(7) ELLIOTT AND BARCLAY SMITH: Journ. Physiol., 1904, xxxi, 281.
(8) WHEELON AND THOMAS: Journ. Lab. Clin. Med., 1920, vi, 137.
(9) ALVAREZ: This Journal, 1915, xxxvii, 267.
(10) ALVAREZ AND STARKWEATHER: This Journal, 1919, l, 252.
(11) ALVAREZ AND MAHONEY: This Journal, 1924, lxix, 211.
(12) DE MEYER: Arch. Internat. de Physiol., 1921, xvi, 64.
(13) ALVAREZ: The mechanics of the digestive tract. New York, 1922.
(14) FRIEDLANDER: Biol. Centralbl., 1888, viii, 363.
(15) LILLIE: Journ. Physical Chem., 1920, xxiv, 165.
(16) KREIDL AND MÜLLER: Pflüger's Arch., 1907, cxvi, 159; MÜLLER AND HESKY: Mitt. a.d. Grenzgeb. d. Med. u. Chir., 1911, xxii, 104; MÜLLER AND KONDO: Ibid., 1912, xxiv, 512.
(17) GASKELL: The involuntary nervous system, London, 1916.
(18) v. UEXKULL: Zeitschr. f. Biol., 1904, xlvi, 400.
(19) FRY: Journ. Physiol., 1909, xxxix, 184.
(20) PARKER: The elementary nervous system, Philadelphia, 1919, 74; POLIMANTI: Arch.. f Physiol., 1915, lxvi, 127; DIXON: Journ. Physol., 1902, xxviii, 66.
(21) SPADOLINI: Arch. d. Fisiol., 1917, xv, 229.
(22) v. UEXKULL: Ergebn. d. Physiol., 1904, iii, part 2, 1.
(23) TRENDELENBURG: Arch. f. Exper. Path. u. Pharm., 1917, lxxxi, 53.

A NON-POLARIZABLE ELECTRODE AND CLAMP DESIGNED PARTICULARLY FOR GASTRO-INTESTINAL WORK

WALTER C. ALVAREZ

From the George Williams Hooper Foundation for Medical Research and the Department of Medicine, University of California Medical School, San Francisco

Received for publication March 29, 1924

When, several years ago, I began to study the action currents of the stomach and bowel, it soon became clear that the first essential was to find a suitable electrode which would follow the peristaltic movements without slipping; which would have a constant potential, and which would stand immersion in Locke's solution. As is well known, any slipping of the electrodes will produce voltage changes. It was a problem also how to fasten the electrodes so that they would not traumatize the wall of the intestine or obstruct its lumen.

At various times during the first two years of work many different types of metallic, and zinc-zinc sulfate electrodes were designed and tested, but none were satisfactory if only because they did not maintain a constant potential. When using a high sensitivity d'Arsonval galvanometer the continual "creeping" was very annoying, and it often made work impossible. Many efforts were made to utilize the various electrodes which have been used by Lewis and others in heart work but the stomach and bowel would not work well for any length of time in a moist chamber. As is well known, the digestive tract works best when kept immersed in a bath of warm Locke's solution. To be sure, electrodes might be fastened on the bowel and the abdomen closed over them but then we would have no means of correlating the mechanical with the electrical phenomena.

Many helpful suggestions as to the use of various types of non-polarizable electrodes were found in articles by Lewis (1), Tigerstedt (2), Mines (3), McSwinney and Mucklow (4), Cohn (5), Williamson (6), McClendon (7), Lund (8), Dryerre (9), Cannon (10), Brown and Hill (11), Eyster and Meek (12), Tschermak (13) and Burker (14). The studies of Ostwald (15) which showed the great constancy of the potential in the calomel electrode led me finally to begin a series of modifications of that instrument. I began first with the Oker Blom (16) apparatus and designed a wooden clamp which held a small loop of bowel against the little opening into which, in the original instrument, a wick is inserted. It

was found that this electrode would work satisfactorily even when Locke's solution was substituted for the usual KCl. This instrument served me well, but as it was unwieldy and unsuitable for many uses, the electrode depicted in figure 1, *1*, was next designed. Much of our work on the stomach has been done with it. It consists of a small bent glass tube with an outside diameter of 5 mm. In one end is sealed a platinum wire which is fused onto the copper wire and that is protected from moisture by some rubber tubing which slips over the glass. Next to the platinum is

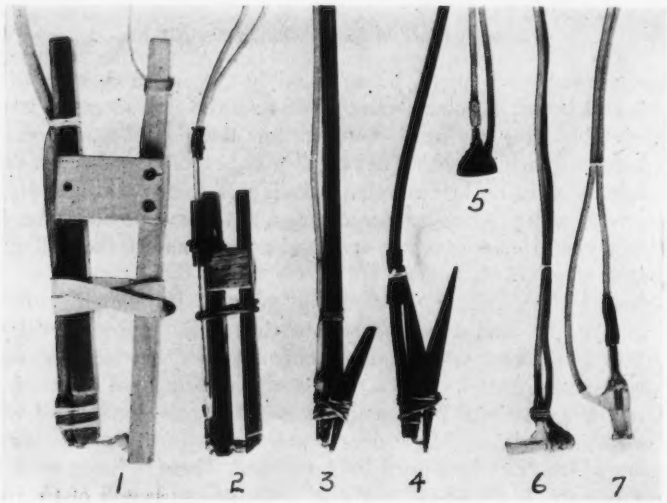


Fig. 1. *1*. Shows a large wooden clamp and electrode used mainly for work on the lesser curvature of the stomach.

2. Small double electrode for intestinal or gall-bladder work.

3. Electrode and clamp for holding immovable a place on the stomach or bowel.

4. Small clamp and electrode most commonly used at present.

5, 6, 7. Various types of suction electrodes. One tube carries the wire and the other maintains suction.

a short column of pure mercury and next to that is a column of mercury calomel paste. This is made by rubbing up pure calomel and pure mercury with a little Locke's solution. The paste is allowed to stand overnight when the excess of mercury settles out. In the larger electrodes a small column of sterile fine sand is placed next to the calomel and that is kept from dropping out by a layer or two of fine butter cloth tied over the slightly flared end of the tube. With the smaller electrodes the sand is omitted and the calomel is held in by a little tuft of cotton. The idea

of using sand was gotten from some work by Coggeshall on the Ostwald electrode (17).

As will be seen from the illustration, the clamp is made of hard rubber or wood boiled in paraffin. The jaws are kept closed with the help of a rubber band. When not in use the electrodes are kept in a beaker of Locke's solution. They keep very well, as will be seen from the fact that the large ones have not been made over for more than three years, and the small ones for over two years. Occasionally the cotton is replaced and perhaps a little more calomel paste is added, but that is all that is needed.

Before using them, we generally test their resistance with a Wheatstone bridge. They usually run around 5000 ohms for the pair, with the ends in contact. During these measurements, if a constant current is used it will be seen that the electrodes are polarizable, but with tiny currents this defect does not seem to be large enough to interfere with our present work.

Figure 1, *2*, shows a smaller type of clamp with two electrodes which we have used on the gall-bladder and bowel. In this instrument the glass is continued up to the top of the clamp and serves to insulate the wire. The two electrodes are 1 cm. apart.

Figure 1, *4*, shows the most convenient model now in use. The glass tube is 3 mm. in outside diameter and is 32 mm. long. The clamp is made out of three pieces of wood and an elastic band. The third piece barely shows in the photograph, but it is a little cylinder 5 mm. long cut from a wooden applicator stick. It fits into a groove cut in each of the little bars of the clamp and serves as a pivot for them. These bars are about 8 cm. long (one has to be longer than the other) and are made of hard wood boiled in paraffin. Figure 1, *3*, shows a similar electrode made with one bar so long that it can be held in a clamp above. In this way one point on a loop of bowel can be fixed. A smaller electrode can be applied nearby and the movements of the bowel can then be recorded simultaneously with the action currents with the help of any one of several technics. A thread may be run from the movable electrode to a tambour connected with a Frank capsule or, when the string galvanometer is being used, the movable electrode may be suspended from one arm of a light heart lever so placed that the other arm will move back and forth in front of the slit of the camera.

Early in the work it occurred to me that the most suitable electrode for work on the stomach would be one which would retain itself in position with the help of suction. I accordingly got my friend, Mr. L. B. Clark, to incorporate the small calomel electrode in a glass cup with a side arm. Suction was applied through fine "spectacle" rubber tubing. Another similar electrode was made by slipping over the electrode one of

the little rubber cups used by dentists for cleaning the teeth. Suction was applied through a little hole in the side of the cup. Good results were obtained with these electrodes but we found that unless the least possible suction was used they soon made the gastric wall very hyperemic, and the character of the electrogastrogram was altered.

There recently appeared a most interesting article by Richter (18) showing his gastrograms obtained with a suction type of zinc electrode. His apparatus would seem to be simpler and better than mine, granting that the voltage is as constant and the upkeep as easy.

For use on the abdomen of man I designed a larger, all-glass electrode 30 cm. long and 1 cm. in outside diameter. The bead of lead glass through which the platinum wire passes was placed 3.5 cm. from the lower end. The upper ends were held in position on the abdomen with the help of ordinary ringstand clamps.

An intra-gastric electrode was easily made by slipping a small glass cylinder into the end of an ordinary Rehfuss type of stomach tube.

One of my friends, after seeing the electrodes in this laboratory, called my attention to a very similar one described by Philippson (19). He uses a straight tube without any clamp. The tube contains mercury, calomel paste and a plug of gelatin or cotton. Another modification of Ostwald's electrode has been designed by Noyons (20). He used a wick to connect the tissues with the calomel.

SUMMARY

A simple, cheap, easily constructed electrode is described. Although designed particularly for use with the digestive tract its reliability and the ease with which it may be used under Locke's solution or tucked away in some body cavity should recommend it to physiologists for general use. Small protecting cups or shields could easily be added to the clamps for nerve work. The suction electrodes and the large tubes for the skin might be used to great advantage in researches on the heart. A short bibliography of articles containing descriptions of electrodes is appended.

BIBLIOGRAPHY

(1) Lewis: Heart, 1910, ii, 149; ibid., 1920, vii, 131.
(2) Tigerstedt: Handb. d. physiol. Methodik, Leipzig, 1911-13, ii, 334.
(3) Mines: Journ. Physiol., 1913, xlvi, 196.
(4) McSwinney and Mucklow: Journ. Physiol., 1922, lvi, 397.
(5) Cohn: Arch. Int. Med., 1920, xxvi, 105.
(6) Williamson: Arch. Int. Med., 1922, xxix, 274.
(7) McClendon: This Journal, 1915, xxxviii, 183.
(8) Lund: Proc. Soc. Exper. Biol. and Med., 1923, xxi, 128.

(9) Dryerre: Journ. Physiol., 1923, lvii, lxiii.
(10) Cannon: This Journal, 1916, xli, 39.
(11) Brown and Hill: Journ. Physiol., 1921, liv, cix.
(12) Eyster and Meek: Arch. Int. Med., 1916, xviii, 777.
(13) Tschermak: Pflüger's Arch., 1919, clxxv, 165.
(14) Barker: Pflüger's Arch., 1900, lxxxi, 78.
(15) Ostwald: Zeitschr. f. physikal. Chem., 1900, xxxv, 333.
(16) Oker Blom: Pflüger's Arch., 1900, lxxix, 534.
(17) Coggeshall: Zeitschr. f. physikal. Chem., 1895, xvii, 62.
(18) Richter: This Journal, 1924, lxvii, 612.
(19) Philippson: Arch. Internat. d. Physiol., 1912, xii, 178.
(20) Noyons: Zeitschr. f. biol. Techn. u. Methodik, 1909, i, 265.

A PHYSIOLOGICAL STUDY OF THE REACTIONS OF YOUNG DECEREBRATE ANIMALS

ORTHELLO R. LANGWORTHY

From the Department of Anatomy, Johns Hopkins University

Received for publication April 1, 1924

The reactions of young decerebrate animals are known to differ considerably from those of adults of the same species. Thus forty decerebrate kittens studied by Weed (12), instead of displaying the intense immobility common to adult animals when deprived of the upper portion of the nervous system, showed not only very active reflexes but many of the younger animals executed prolonged progressive movements. Previously Graham Brown (5) had reported alternate progression in one litter of fetal kittens after the cortex and basal ganglia had been removed. The mechanism controlling progression seems therefore to develop relatively early in cats while a tendency toward extensor rigidity seems not to be present at birth but to be developed at an early period in extra-uterine life. With the data at hand we may hypothesize a reciprocal relationship between the activity of young decerebrate animals and the onset of decerebrate rigidity. It was with the purpose of further testing this theory and also of studying the time of onset of rigidity in young animals of other species that this work was undertaken.

Sherrington (7) was the first to describe the phenomenon of decerebrate rigidity appearing in adult animals after removal of the cerebrum and basal ganglia. In this procedure the brain stem is usually severed just anterior to the superior colliculi, the line of transection, of course, varying slightly in individual cases. As the anesthesia wears off the contraction of the extensor muscles first becomes manifest in the elbow joint and knee. Later the shoulder, hip and ankle are affected. The contraction once developed continues as long as the animal remains in good condition and may be roughly estimated by the strength of pressure necessary to overcome the spasticity. Sherrington (9) considered the condition to be essentially the reflex posture of standing, the muscles affected being essentially those acting against the force of gravity.

Descriptions of the general conduct of such decerebrate animals have been given by Sherrington (8), Graham Brown (3) and Weed (11). Bazett and Penfield (1) observed the reactions of the animals over periods of many days, keeping them alive as chronic preparations. As the heat-regu-

lating mechanism had been injured, it was necessary to suspend them in baths at body temperature. Some of the animals lived for a considerable period but hemorrhages and degenerations in the brain stem clouded the picture and made their behavior difficult of interpretation.

Magnus and de Kleijn (6) reported a few instances of the development of decerebrate rigidity in man following pathological processes in the brain. One new-born infant showed typical decerebrate rigidity particularly in the legs following an extensive hemorrhage into the ventricles destroying the decerebral radiation above the midbrain. The arms in man appear to be somewhat released from decerebrate control.

The forty kittens described by Weed ranged in age from one hour to fifty-seven days. One of the first reactions to this decerebration that could be demonstrated was withdrawal of the leg on pinching the foot or foot pads. The younger of the kittens were by far the most active in this regard, a mere touch to the foot pad serving as an adequate stimulus. Such a pulling away of the leg in adult preparations can be demonstrated only after very severe trauma of the foot. The reactions to trauma applied to the tail were also more outspoken than in the adult. A slight pinch of the tail between the fingers caused a bilateral thrust of the hind legs or in the more active kittens several rhythmic thrusts,—typical leaping movements. All the kittens exhibited scratch reflexes. They presented different activities at different times during the experiment. Thus the period of observation was divided into three portions, the first when the animal was just recovering from the anesthetic, the second during the maximum reflex activity and the third during the period of respiratory distress and decline.

If during the period of optimum activity these kittens in Weed's series were subjected to stimuli which in the intact cat would cause pain, they responded by making characteristic walking movements of all four legs. Such progressive movements had been obtained in the adult cat with maximal stimulation (Sherrington and Woodworth (10)) but they were of short duration. In Weed's animals excitations of very slight degree were often sufficient for their inauguration. But the most important feature of these progressive movements was that in twelve of the more active kittens they continued for a considerable period (at least over thirty seconds) after removal of the stimulus. Several of the animals walked till they were exhausted.

Almost any kind of stimulus reaching the intact portion of the brain stem was sufficient to initiate these walking movements. Thus in certain of the animals loud auditory stimuli would start progression wholly similar to the walking or running movements seen in normal adult cats.

The animals exhibiting this prolonged progression were, in general, the younger, eight of the twelve being less than five days old. It is interesting

to also note that only five of the twelve showed any tendency to extensor stiffness and in three of these the rigidity was extremely doubtful. All of these five animals exhibited rigidity only in the fore legs. The rigidity when present appeared immediately after the anesthesia had worn off and disappeared permanently after the initiation of progression. Removal of the cerebellum in two of the animals permanently stopped progressive movements.

Nineteen of the other twenty-eight animals made progressive movements of all four legs quite similar to those of the twelve more active kittens, never continuing, however, more than thirty seconds after removal of the stimulus. The method of stimulation differed only in the greater degree of excitation required. Of the nineteen animals belonging to this group only four were less than ten days old; six were between the tenth and twentieth day. It is very interesting to note that twenty of the twenty-four kittens of the series in which rigidity was noted were over ten days old. The nine kittens of the group not exhibiting true progression showed in general rhythmic activities of either the fore or hind legs. No typical coördinated walking movements could however be obtained.

Several litters of kittens were reported by Weed and the members studied at various ages. The results give a very remarkable picture, the younger animals displaying prolonged progression which in older animals was replaced by extensor stiffness present first in the fore, later in both fore and hind legs.

I. THE REACTIONS OF TEN OTHER DECEREBRATE KITTENS. To serve as a normal control for the author in his study of the reactions of other animals, ten more decerebrate kittens were studied. These animals varied in age from three hours to twenty-one days. The operation performed and the subsequent precedures were similar to those described by Weed. It is needless to say that the animals were kept completely anesthetized until after the operation was complete. However, it was necessary to administer ether with the greatest care for the slighest overdose of anesthetic severely interfered with the development of the reflexes. The plane of transection of the neuraxis was constantly through the body of, or just anterior to, the superior corpora quadrigemina.

The animals showed several reflex phenomena in common. Thus pinching the foot pads would, in every case, cause withdrawal of the leg in active flexion. The strength of stimulus necessary to evoke this reaction varied in general inversely with the age of the animals. They also showed extensor thrusts of the hind legs when the tail was lightly pinched between the fingers. The more active kittens reacted with a series of extensor thrusts continuing after cessation of the stimulus and sometimes accompanied by alternate beats of the fore legs. The animals also exhibited a well-developed and precise scratch reflex.

One other reflex was constantly present. The smoke of a cigarette placed under the nose caused a turning of the head to one side with convulsions of the facial musculature preceding a sneeze or a series of sneezes. The area of the brain presiding over this reflex therefore lies below the plane of transection. Many of the kittens uttered low cries when stimulated vigorously.

The reaction of greatest interest however was the occurrence of progression in these young decerebrate animals. In order that they might be grouped in accordance with their relative activities a persistence of walking movements for at least thirty seconds after removal of the stimulus was considered prolonged progression. Seven of the ten animals studied were less than ten days old. Of these seven kittens, five showed typical long-continued progressive movements. One of the other two never breathed well; the other was decerebrated after previous removal of the cerebral hemispheres when in poor condition. Prolonged progression was also exhibited by one of the three older kittens, thirteen days old.

The characteristics of this progression as given by Weed have practically all been verified in this small series. Thus almost any sensory stimulus applied to any part of the body would initiate walking movements during the period of maximal activity. Merely placing the fingers under the foot pads was often an adequate stimulus. One kitten reacted to loud noises. The irritation of the strings by which they were suspended started in several animals spontaneous diagonal movements. Three of the kittens progressed until exhausted nor did it seem possible to stop them. One of the younger animals when placed upon the floor walked in a sprawling manner slightly faster than was possible before the decerebration. Placing a smooth surface beneath the feet of the walking animals has a tendency to slow the beats somewhat, but it serves often to initiate the movements.

One of the kittens showed poor coördination of the rhythmic movements, the hind and fore legs beating at different rhythms. Another while exhibiting rhythmic beats of the fore legs showed a tendency at times for the hind legs to be brought up in a typical scratch reflex—a phenomenon possibly of asphyxial origin.

Two kittens of the series, one thirteen and the other twenty-one days old, remain to be described. These two animals exhibited progressive movements on rather strong stimulation, not continuing, however, for any length of time after removal of the stimulus. The older of these animals showed another unique phenomenon of the irritative type. When lying upon the floor it placed the right fore leg tightly against the head and arching its back rolled over and over toward the left, indicative of possible injury to the vestibular mechanism.

Three of the animals under ten days old exhibited extensor stiffness appearing as the anesthetic wore off and disappearing at the initiation of

walking movements. In two of the animals the rigidity was only apparent in the fore legs; in the third it was also present in the hind legs. A fourth animal showed adduction of the fore legs but no resistance to passive flexion. All three of the animals over ten days old showed rigidity in both fore and hind legs.

The results of this small group of experiments upon decerebrate kittens thus substantiate the work previously reported by Weed, the tendency toward prolonged progression being well developed in new-born animals with a gradual development of extensor stiffness at a slightly later period.

II. THE REACTIONS OF TWENTY-TWO YOUNG DECEREBRATE RABBITS. It seemed particularly interesting to perform similar experiments upon two different types of animals, one in which the young were born after a short intra-uterine development in a very immature condition and another in which the young were born extremely late at a time when they were almost able to cope independently with their environment. Two common laboratory animals were found to meet these qualifications, the rabbit was chosen as an example of the first, the guinea pig as an example of the second type.

Rabbit embryos are carried in utero but thirty days and are born at the end of this time in a very immature condition. No coat of fur has yet developed, the skin being covered only by traces of a fine lanugo-like hair. The eyes are closed. Ordinarily the first trace of down appears on the body about the tenth day and about the twelfth day the eyes open. The length of the new-born animals varies from 100 to 110 mm. After birth they normally grow very rapidly. It seemed therefore possible that the phenomenon of decerebrate rigidity might develop relatively late in these immature animals and that in the meantime the decerebrate preparations might be very active.

The nineteen young rabbits in this series varied in age from new-born to thirty-four days old. In addition three fetal animals were also studied. The youngest animal was 70 mm. in length, the oldest, 235 mm. The latter was almost half grown, had been weaned and was quite able to care for itself.

Three fetal rabbits, 70 mm. in length, were born prematurely and brought to me immediately. When received they were already cold and their respirations were slow and irregular. In fact they appeared to be in a moribund condition. When placed in the warm box their general condition improved somewhat but they were very sluggish, unable to move about and reacted slightly even to strong stimulation. When they had improved as much as could be expected they were decerebrated under ether and suspended in a warm box.

As the anesthetic wore off the animals began spontaneous progressive movements continuing in one over twenty, in the other two over forty-five

minutes or until they were exhausted. These movements strangely enough showed an alternating rhythm rather than the hopping type of rabbit progression principally noted in the other preparations of this series. The legs showed throughout the experiment rather poor cöordination. The beats were at first relatively slow and could be further slowed and subsequently greatly accelerated by the application of nocuous stimuli. The rate of progression had a tendency to become much more accelerated after the first few minutes. The animals finally became so active that they swung their bodies back and forth on the strings by which they were suspended. When a smooth surface was placed under their feet the rate became markedly slower. The animals at no time showed any ability to support themselves upon their legs.

When they were at the height of their activity it was impossible to restrain them or to decrease the tremendously active progression. But as exhaustion set in, the beats became progressively slower and finally ceased. Then with an adequate stimulus progression could again be initiated but only for short periods of time.

As they became quiet it was possible for the first time to adequately test the activity of other reflexes. Then it was found that pinching the foot pad would cause flexion of the leg with extension of its contralateral fellow. Also slight pinching of the tail caused a series of bilateral extensor thrusts of the hind legs often accompanied by alternate beats of the fore legs or followed by a period of progression. Pinching the tail during the period of maximal activity would often temporarily initiate the hopping progressive rhythm. All three animals exhibited a scratch reflex. We have here therefore three very active premature decerebrate preparations showing violent and poorly coördinated progression of a different type than that observed in rabbits after birth.

Nineteen other animals were born at term and studied at various periods thereafter. Four were less than one day old, ten less than ten days, six between ten and twenty and two were twenty days of age.

Thirteen of these preparations showed after decerebration typical prolonged progressive movements, persisting continuously in some of the more active animals for over an hour. The walking movements differed in several respects from those observed in kittens. The intensity of the reflex and the persistence with which it was continued in these tremendously active animals was almost incredible.

A number of the mothers took very poor care of their offspring and the younger animals of the series were often brought into the laboratory cold and almost lifeless. Very often the respirations were slow and irregular. Warming the young frequently improved their condition somewhat. But even when they were warm and well cared for they lay quietly and responded very little to stimulation. They were unable to support any of

their weight upon their legs. Pinching a foot pad would cause withdrawal of the leg and possibly initiate a few rhythmic movements of all four legs which, however, were entirely purposeless in character and soon subsided.

Decerebration caused a tremendous change in the reaction picture of these animals. During the process of suspension rhythmic movements of the legs usually began spontaneously, to continue over long periods of time. The rhythmic movements of the rabbits were in general not of the alternating type but rather a hopping type of progression characteristic of the healthy adults. Thus bilateral extensor thrusts of the hind legs were combined with synchronous alternating beats of the fore legs. In many of the animals this hopping progression was not continuous but was at times replaced by the trotting type of limb movement, as was noted in the three immature animals already described. In two of the younger animals the progression was poorly coördinated, the fore legs beating at almost twice the rate of the hind legs.

The progressive movements of the rabbits were more intense and more violent than those observed in kittens. Thus when the younger animals were particularly active they swung back and forth upon the strings that suspended them. The rate of the rhythm was also often much faster than any observed in the kittens. Nor was any particular stimulus required to initiate the hopping reflex, the mere act of suspending the animals initiated movements continuing uninterruptedly for long periods. After this period of activity was over no stimulus seemed adequate to produce progression of very long duration.

Rabbits in general were found to react very well to decerebration. Thus in none of the animals did we have to deal with respiratory distress early in the experiment. For this reason the observations seem particularly clear cut.

All of the eight animals under ten days of age as well as five of the six between ten and twenty days old exhibited prolonged progression. The rhythmic tendency, however, seemed to be less marked in the older members of the group. Thus three preparations over ten days old showed progression not lasting over two minutes and requiring rather intense stimulation to elicit it.

The three other animals belong to the short enduring reflex group, progression lasting less than thirty seconds after removal of the stimulus. They were able to support the weight of their body upon the feet and when the fingers were placed under their feet well coördinated attempts at balancing could be noted. All of these animals showed a well-developed extensor rigidity which evidently dominated over the progressive tendency.

None of the eight animals under ten days old showed any extensor rigidity whatever; nor were the legs at rest placed in a position of extension and adduction, the first hint of the development of this phenomenon.

Three of the six animals between ten and twenty days old showed definite extensor stiffness of the fore legs, one also exhibiting a definite resistance in the hind legs to passive flexion. Two other animals showed definite extension and adduction of the fore legs without any apparent resistance to passive movement while the sixth showed no tendency toward rigidity at all. The extensor rigidity when present usually appeared as the animals recovered from the anesthetic and disappeared permanently upon the initiation of progression. The two animals of the series over twenty days old showed well-developed and maintained rigidity in both fore and hind legs.

All the rabbits of the series when decerebrated showed the standard group of reflexes already described in the kittens. Thus the scratch reflex was easily obtained in all the animals. They also gave bilateral extensor thrusts of the hind legs when the tail was pinched. Stimulation of the foot pads of one of the fore legs caused flexion of that leg and extension of the contralateral leg. Stimulation of the foot pad of one of the hind legs often caused bilateral extensor thrusts of both hind legs.

The sucking reflex was also obtained in six of the younger animals. Thus stimulation of the area around the lips with a blunt instrument caused pursing of the lips with typical sucking movements. Gentle stroking of the dorsal surface of the body gave in the younger animals well marked ventral bowing of the back; similarly ventral stimulation gave a dorsal bowing. The animals frequently uttered cries when stimulated.

As the animals began to exhibit respiratory distress, a new asphyxial type of progression very often appeared. This differed in many ways from the rhythmic activities noted earlier in the experiments and was in no way confusing.

One litter of six rabbits was kept alive in the laboratory and the young decerebrated at different ages. The young when killed were four, thirteen, fifteen, nineteen, twenty-six and forty-two days old. The preparation made at the end of four days showed prolonged progressive movements with no trace of extensor rigidity. The one thirteen days old showed short periods of progression with some rigidity in the forelegs. The preparations fifteen and nineteen days old made prolonged progressive movements with development of rigidity in the fore legs only of the one nineteen days old. The two older animals developed rigidity both in the fore and hind legs with progression only of the short enduring type.

III. The reactions of seventeen young decerebrate guinea pigs. The guinea pig young were chosen as experimental animals because of their precocious activity immediately after birth. The period of gestation in the guinea pig approximates two months and the young are born in a relatively mature condition. Thus at birth they have a well-developed coat of fur and their eyes are open. They can support themselves upon

their legs and run almost as fast as adults. They immediately begin to eat some solid food. It seemed very possible therefore that the neurological mechanism counteracting the force of gravity should be well developed at birth in these animals.

Seventeen experiments were conducted in this group, four of the animals being less than one day old and all seventeen less than ten days. It is a striking fact that in none of these animals prolonged progressive movements could be induced. Short enduring progression was obtained in eight of the animals while in the others rhythmic activity did not continue after removal of the stimulus. Moreover, a nearly maximal stimulus was required to elicit this reflex.

We find on the other hand an early development of extensor rigidity. Fourteen preparations showed decerebrate rigidity in the fore legs. In eleven of these there was present rigidity in the hind legs. Extension and adduction of the forelegs was noted in two of these animals not showing resistance to passive movements. The third animal was breathing poorly and died early in the experiment.

The young guinea pigs in general showed that degree of immobility so characteristic of adult preparations. Extremely strong stimuli were necessary to obtain any reflex reaction at all. It was possible to elicit the scratch reflex in only seven animals of the series. Maximal pinching of the skin over the caudal area of the body usually caused bilateral extensor thrusts of the hind legs. The animals frequently cried out when stimulated.

IV. THE REACTIONS OF TWO DECEREBRATE PUPPIES. Two young puppies were obtained for examination and were decerebrated at the age of two and of five days. Definite rigidity was observed in both fore and hind legs of these animals. Short enduring periods of progressive activity followed strong stimulation; the rhythm was always of the alternate type. Movements of the legs were poorly coördinated in the younger of the preparations, the hind legs showing a slower rhythm than the fore.

V. RHYTHMIC MOVEMENTS OF THE HIND LEGS FOLLOWING A TRANSVERSE SECTION OF THE THORACIC SPINAL CORD IN YOUNG DECEREBRATE ANIMALS. Another group of observations was made upon the young animals suggested by previous work of Graham Brown (2), (4). He found that if the thoracic cord was quickly cut transversely in the adult decerebrate cat, the hind legs were first sharply flexed and then executed a number of rhythmic beats ending in extreme extension. This phenomenon took place even after the hind legs had been completely deafferented. Brown suggested that rhythmic progression was conditioned by the equal balance of antagonistic central activities not fundamentally of peripheral origin. The phenomenon of progression evoked by rapid division of the thoracic spinal cord might be produced by mechanical stimulation in the

cord of descending fibers, some of which give the flexion stimulus and some the extensor stimulus, causing a momentary disturbance of equilibrium between these two systems. He hypothesized therefore a balance between flexion and extension lying completely on the motor side but ordinarily regulated by afferent impulses from the periphery. This motor mechanism may be stimulated by substances carried in the blood stream. Thus narcosis progression occurs in cats at a depth of anesthesia at which the reflex arc and tracts in the cord are no longer functional.

It seemed interesting to note therefore whether rhythmic movements could be observed in the hind legs of these young decerebrate animals after transection of the spinal cord in the thoracic region. As this experiment could only be carried on after the other observations were complete the series is not a large one.

Eleven rabbits were examined varying in age from one to eighteen days. In all of these animals the results were practically the same. The spinal cord was cut quickly with one snip of the scissors. Immediately the hind legs were sharply flexed so that they lay tightly against the abdomen. The legs then executed ten to twenty rhythmic beats becoming more and more extended so that they finally came to rest in a position of extreme extension. No further movement occurred. The hind legs were entirely flaccid. The rabbits of various ages all gave the same picture. Similar results were obtained from two kittens, thirteen and twenty-one days old.

This phenomenon could not be observed, however, in any of the six guinea pigs upon which it was tried. After cutting the cord in these animals sharp flexion of the hind legs was followed by gradually developing extension without any intermediate beats.

SUMMARY

Weed (12) has previously shown that kittens if decerebrated during the first few days of life have often very active reflexes and show a tendency toward prolonged progressive movements. In slightly older animals the phenomenon of extensor rigidity develops and the preparations are far less active. The present paper reports the results of decerebration in other kittens and also in young rabbits, animals born very immature, and in young guinea pigs, animals born so mature that they can almost care for themselves.

Twenty-two young rabbits varying in age from one hour to thirty-four days were decerebrated and their reactions studied. The younger preparations were tremendously active, exhibiting prolonged progressive movements beginning often spontaneously as the animals recovered from the anesthetic and sometimes continuing over an hour. No rigidity was present. The phenomenon of decerebrate rigidity appeared in the older

animals and at the same time their progressive activity was greatly curtailed. Decerebrate rigidity begins to develop at about the time that the animals are first able to support their weight up on their legs.

Guinea pigs at birth are able to run about almost as actively as adults. All but one of the seventeen decerebrate preparations, including several animals less than one day old showed signs of decerebrate rigidity, the rigidity being well developed in a majority of the animals. Nor did any of the preparations display any tendency toward prolonged progressive movements.

If the spinal cord was cut transversely in the thoracic region in young decerebrate rabbits, flexion of the hind legs was soon followed by a number of rhythmic beats, the legs finally coming to rest in a position of extreme extension. This phenomenon has been observed by Graham Brown in adult decerebrate animals even after the hind legs have been completely deafferented and formed the basis for an interesting hypothesis concerning the balance of flexors and extensors in the limb. It is interesting to note that this phenomenon is already active in young animals.

BIBLIOGRAPHY

(1) Bazett and Penfield: Brain, 1922, xlv, 185.
(2) Brown: Proc. Roy. Soc., 1912, lxxiv, 308.
(3) Brown: Proc. Roy. Soc., B., 1913, lxxxvii, 145.
(4) Brown: Journ. Physiol., 1914, xlviii, 18.
(5) Brown: Journ. Physiol., 1915, xlix, 208.
(6) Magnus and de Kleijn: Pflügers Arch., 1912, cxlv, 455.
(7) Sherrington: Proc. Roy. Soc., 1896, lx, 414.
(8) Sherrington: Journ. Physiol., 1898, xxii, 319.
(9) Sherrington: Brain, 1915, xxxviii, 191.
(10) Sherrington and Woodworth: Journ. Physiol., 1904, xxi, 234.
(11) Weed: Journ. Physiol., 1914, xlviii, 205.
(12) Weed: This Journal, 1917, xliii, 131.

REACTIONS OF INTESTINAL SEGMENTS TO MECHANICAL STIMULATION

R. G. HOSKINS AND EDGAR S. HUNTER

From the Department of Physiology, Ohio State University

Received for publication April 5, 1924

Numerous observers have called attention to the fact that smooth muscle preparations, and especially intestinal segments, are often highly sensitive to mechanical stimulation. Grützner (1) emphasized this in his Ergebnisse article on smooth muscle physiology in 1903. In connection with a study of the relations of oxygen and adrenalin to the activities of intestinal segments (2), the factor of incidental mechanical stimulation often introduced a disturbing variable. In particular it was noted that the administration of oxygen by bubbling the gas through the fluid surrounding a muscle segment may produce a greater effect by its mechanical action than as oxygen. It seemed desirable, therefore, to make a systematic study of the reactions to mechanical stimulation per se.

In the experiments herein reported the white rat was used ordinarily as a source of material. Both adults and half-grown animals were employed. In order to avoid the possible disturbing factor of the estrual cycle males were used for the most part, but owing to a shortage of animals at one stage of the investigation females were also employed. So far as our experience goes to show, however, the sex cycle is not a significantly disturbing factor. A few supplementary observations were made upon segments of adult male guinea pigs, but these proved less satisfactory than those from rats. Segments from the duodenum, the jejunum and the ileum were studied. In a general way evidence of a "metabolic gradient" as set forth by Alvarez (3) was seen, but the decrement of activity in lower as compared with higher segments was by no means a constant finding. Longitudinal segments only were used.

A satisfactory mechanical stimulus was obtained by connecting the "buzzer" of a Harvard inductorium by means of a silk thread to the string by which the segment was attached to a light writing lever. Usually two preparations were studied simultaneously, both being stimulated from the same inductorium. In most cases twenty seconds but in a few instances thirty seconds was the period of stimulation.

The segments were immersed in beakers holding 50 cc. of mammalian Ringer's solution. These were kept in a water bath holding approximately

40 liters. The water bath was heated by two Bunsen burners controlled by a glycerin thermostat. The temperature varied on different occasions from 35° to 37°, but in any given experiment usually remained constant within half a degree. The Ringer's solution was made up of NaCl 0.9 per cent, CaCl 0.024 per cent, KCl 0.042 per cent, $NaHCO_3$ 0.02 per cent, in water once distilled in glass. After storage in glass this had a pH of approximately 8 (thymol blue and phenol red indicators).

The form of the reaction to mechanical stimulation varied markedly in different preparations and in the same preparation at different times. Figure 1 illustrates most of the types encountered. The most common form of reaction was a relaxation succeeded by marked augmentation.

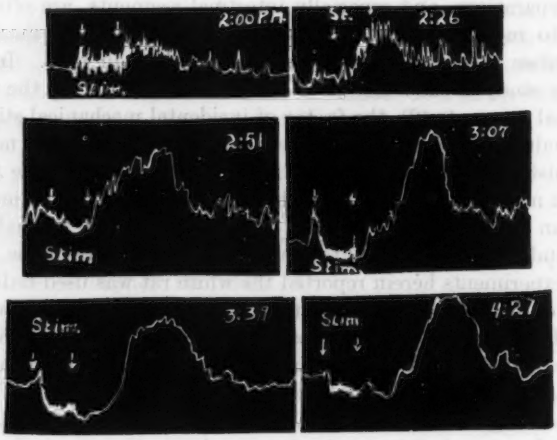


Fig. 1. Segment of duodenum 2 cm. long from male adult rat. Stimulated mechanically for 20 seconds. Graphs selected at intervals to show progressive variations in form of reactions.

With highly irritable preparations the first effect was an increase in the height of the rhythmic contractions, and somewhat later an increase in the tonus. The former reaction rarely out-lasted the period of stimulation, but the increased tonus ordinarily persisted from one to two minutes. In the majority of cases the rhythmic contractions were little affected. As the stimulation was repeated at three to five minute intervals the form of the reaction usually changed in a rather striking way. Instead of a pure increase in tonus there was first a slackening which persisted in various instances from ten to twenty seconds. This was succeeded by an increase of the tonus of varying degrees. The latent period for the tonus reaction was usually very short, often no more than one or two

seconds, but a latency of as much as thirty-five seconds might intervene. Perhaps the most characteristic type of reaction is a preliminary dip in tonus, followed by a rise beginning during the period of stimulation and lasting about one minute, to be followed by a return to the initial tonus level in the course of another minute. Such a reaction is shown in figure 4 at *3:40*. In a few cases the augmented tonus persisted two to three minutes. Evans and Underhill (4) have stated that mechanical stimulation characteristically causes relaxation when the preparation is in higher tonus and contraction when the initial tonus is lower. When the tonus changes were occurring fairly rapidly due to variations in oxygen supply our observations bore out this statement. On the other hand, when the reactions were obtained from different tonus levels coming about more slowly through gradual "spontaneous" relaxation of the preparation the reactions were more often of the same general form. In almost every instance some degree of tonus increase followed stimulation if the preparation was in any degree reactive. Figure 2, however, shows a case in which only relaxation was obtained.

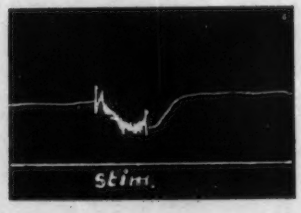


Fig. 2. Segment of jejunum from female rat weighing 120 grams. Stimulated mechanically for 20 seconds. Graph shows pure depression of tonus.

The administration of oxygen to the tissues by allowing it to bubble into the container through a capillary pipette often resulted in an increase of irritability to mechanical stimulation even after the apparent effect of the oxygen had ceased. In other cases, however, as shown in figure 3, the oxygen had little or no effect on irritability to mechanical stimulation. Of the two types of stimulation, the oxygen almost always produced the greater effect (fig. 3). In one instance, however, *3:17*, figure 4, the tissue was almost insensitive to oxygen, but within three minutes was found to respond vigorously to the mechanical stimulation.

No systematic study was made of the effects of variations in temperature on the reactivity. In one instance, however, the gas accidentally flashed back in one of the Bunsen burners and within a period of seventeen minutes the temperature dropped from 35.6° to 35°. This fortunately occurred at a time when the preparation was in the relatively constant phase of its reactivity. In this instance the change of 0.6 of a degree resulted in no significant variation in the reaction.

A striking feature noticed was the marked variability of the different segments of the intestine of a given animal. Despite the fact that the temperature and composition of the surrounding medium were the same there was no close resemblance in the behavior of even immediately adjoining segments, either as regards spontaneous activity or reactions

to stimulation. The variability in such cases was occasionally as great as 100 per cent despite the fact that the segments were of the same length and were manipulated and stimulated in as nearly as possible an identical fashion.

The question with which we were particularly concerned was whether, in the face of the variability indicated in the foregoing paragraphs, conditions could be sufficiently controlled to permit comparison of the reactions before and after the administration of adrenalin. A study was therefore made of the course of reaction changes over considerable periods of time. In case of not a few preparations the behavior was quite unpredictable from one stimulation to the next. In a very considerable proportion of the

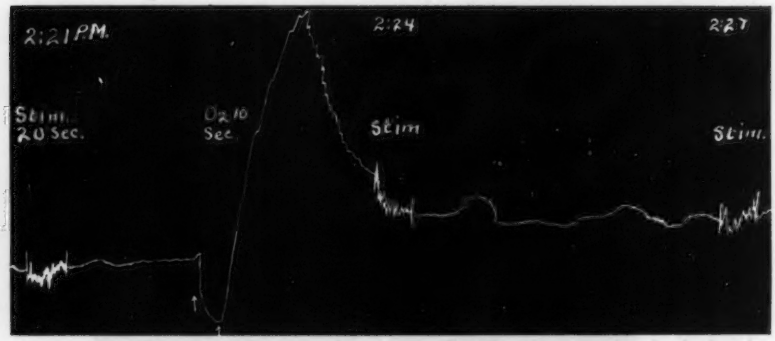


Fig. 3. Segment of upper ileum 2.5 cm. long from female rat weighing 120 grams. Stimulated mechanically for 20 seconds at points indicated. Oxygen administered by bubbling for 10 seconds between first and second stimulation. Graphs show high irritability to oxygen, low irritability to mechanical stimulation and failure of oxygen to increase sensitivity to mechanical stimulation.

cases, however, the reactivity passed through a strikingly regular evolution. Figure 4 will serve to illustrate this. From a relatively refractory condition (in some cases absolutely refractory) the preparation progressed through a stage of increasing irritability, reached a fastigium and gradually declined to a point of little or no reactivity. The period of such evolutions varied within rather wide limits. In some instances the evolution was practically completed within fifteen three-minute periods. In other cases the period was longer. In one instance the preparation was still giving a reaction about half as extensive as the greatest reached when the experiment had to be discontinued at the end of two and one-half hours. As a practical procedure, therefore, it proved to be necessary to make an individual study of each segment through a period of ten or more stimulations. It was found that if the evolution is symmetrical through this

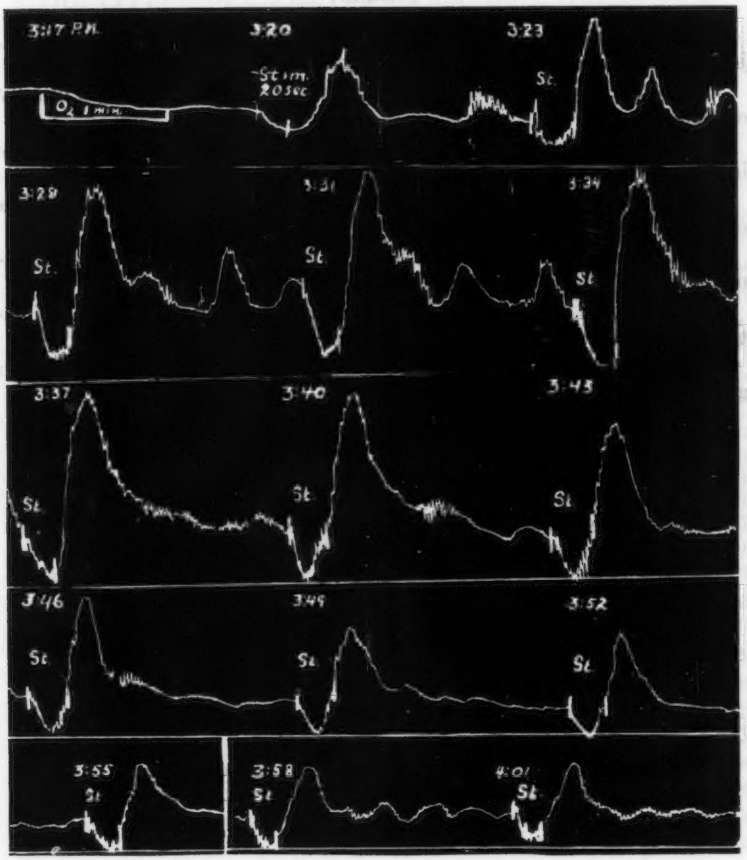


Fig. 4. Segment of upper ileum 2.5 cm. long from female rat weighing 120 grams. Graphs show characteristic evolution of type of reaction to mechanical stimulation.

period one can count with a high degree of probability upon its continuing regular in its reactions for a sufficient period to permit valid comparative observations before and after the introduction of an experimental variable.

SUMMARY

1. Longitudinal segments of rat and guinea pig intestine (duodenum, jejunum and ileum) were stimulated mechanically.

2. Rhythmic contractions were occasionally and tonus changes usually evoked.

3. The type, latency, extent and persistence of reaction varied markedly in different segments as well as in the same segment at different times.

4. The most common type of reaction was a brief fall followed by a considerable rise in tonus.

5. Administration of oxygen frequently but not invariably caused augmentation of the reaction to mechanical stimulation.

6. The tissues were usually but not always more sensitive to administration of oxygen than to mechanical stimulation.

7. A considerable proportion of the individual segments were found to go through a regular evolution of reaction types.

BIBLIOGRAPHY

(1) GRÜTZNER: Ergebn. d. Physiol., 1903, iii, 2, 60.
(2) HOSKINS AND HUNTER: Journ. Pharm. Exper. Therap., 1924 (in press).
(3) ALVAREZ: Mechanics of the intestinal tract. New York, 1922.
(4) EVANS AND UNDERHILL: Journ. Physiol., 1923, lviii, 1.

THE EFFECT OF INGESTED ADRENALIN CHLORIDE ON BASAL METABOLISM

F. A. HITCHCOCK

From the Laboratories of Physiology, Ohio State University

Received for publication April 5, 1924

Adrenalin chloride has frequently been administered to patients by mouth in the belief that it would be absorbed and exert some systemic effect. The clinical evidence of its efficacy is by no means convincing and physiologists and pharmacologists have, as a rule, maintained that there is little or no absorption of this substance from the upper gastro-intestinal tract. It need scarcely be pointed out that local effects could conceivably be produced in the stomach and intestines without absorption into the blood stream, though such a differential absorption seems by no means likely.

The opposing views are reflected in recent textbooks and periodical literature. Hatcher and Wilbert (1) state that "epinephrin is one of the few alkaloids in the materia medica which are not absorbed from the gastro-intestinal canal with such rapidity as to induce any appreciable effect." Bastedo (2) makes the more conservative statement that "as a rule no systemic effect at all is obtained from the drug (epinephrin) when it is given by mouth or applied to mucous membranes." Funk (3), without discussing the absorption of the drug, states that the dose of adrenalin chloride to be given internally is from five to ten minims. Rowntree (4) has quite recently reported several cases of Addison's disease which were treated, with some degree of success, by the use of epinephrin hypodermically and by rectum, and of desiccated suprarenal extract by mouth. In evaluating such data, however, the remissions that are often seen in this disorder offer a serious difficulty. The fact that a patient's symptoms improve after administration of epinephrin is paralleled by the fact that they not infrequently improve without any treatment whatever. Trias and Dorlencourt (5) reported that the ingestion of 0.3 mgm. of epinephrin per kilo of body weight in dogs was followed by hyperglycemia. They draw the conclusion, therefore, that there is some absorption from the stomach. They also stated in the same paper that the absorption varies directly with the concentration of the drug in the stomach, and occurs more readily if the epinephrin solution is rendered isotonic. Rowntree (4) states that the whole gland, given by mouth in excess

causes intestinal disturbance and cramps. However, Bastedo (2) makes the statement: "It is reported that an aqueous extract of two pounds of the fresh suprarenal capsules has been swallowed without ill effects." Danielopolu and Carniol (6) have shown that the introduction of 1 mgm. of adrenalin into the human stomach produces a marked exaggeration of the gastric contractions.

Vincent (7) sums up the situation as follows:

> It is well to recall that adrenal extracts were first administered by subcutaneous injections. But since Oliver and Schäfer reported that the activity of the gland is not impaired in vitro by pepsin and hydrochloric acid, it has become customary to give it by mouth. . . . . Some years ago the present writer failed to observe any physiological effects upon dogs, cats, and rabbits, of feeding with the adrenal bodies of sheep, and the administration of large doses of extracts (in some cases made from the medulla only), failed to produce any noticeable rise of blood pressure in the human subject. But Leyton states that adrenal extract, although when administered by mouth, it ordinarily fails to produce elevation of blood pressure, will bring about this effect in cases of Addison's disease. However this may be, the most certain method by which to obtain any definite pharmacodynamical effects is that of subcutaneous, intramuscular, or intravenous injections.

That pharmacologists have usually obtained negative results from adrenalin by mouth might, of course, be due merely to the fact that the tests employed were not sufficiently delicate. The recent work of Boothby and Sandiford (8) has shown that adrenalin chloride administered subcutaneously in doses as small as 0.5 mgm. is sufficient to cause well-marked increase in basal metabolism in adult human subjects. In view of the well-known slowness of absorption of the drug under this condition such increase shows that it may act effectively in very minute concentration. It seems desirable, therefore, to reinvestigate the utility of oral administration of adrenalin, using the change of basal metabolism as a criterion. It was assumed that a rise in the metabolic rate after the ingestion of adrenalin chloride may be taken as an indication of absorption from the stomach and that the extent of the rise may be taken as a rough measure of the amount absorbed.

Method. Men ranging in age from 19 to 35 years were used as subjects. Except in three cases the tests were made in the morning, the subject coming to the laboratory without breakfast. In the other three instances the subject ate a light breakfast at about 7:00 a.m. and reported at the laboratory for the determination at about 2:00 p.m., having taken no food in the interval. The results obtained indicate that the effects of the breakfast had worn off before the beginning of the experiment. After a rest period of approximately thirty minutes the expired air was collected for a period ranging from eight to twelve minutes. In some cases a placebo, consisting of chloretone dissolved in 0.7 per cent NaCl, was then given and a second collection made. Sometimes, however, the placebo

and the normal control were combined in one test, and in one case the placebo was omitted. The subject was then given adrenalin by mouth. The dose used varied from 3 to 10 cc. of Parke Davis' 1:1000 solution of adrenalin chloride, diluted to a total volume of 100 cc. After the ingestion of adrenalin from one to three determinations of the metabolic rate were made, at intervals of about fifteen minutes.

The expired air from the subject was collected in a Tissot spirometer by means of a face mask similar to that described by Boothby and Sandiford (9). Henderson's modification of the Haldane air analysis apparatus (10) was used. The analyses were all made in duplicate, and only determinations in which the duplicate analyses checked within 0.08 per cent are considered in the results. As regards details of manipulation and calculation, the methods of Boothby and Sandiford (9) were closely followed. The basal metabolic rate was calculated to the DuBois normal standards and the DuBois formula for body surface area was employed.

In most of the experiments the subject breathed out-door air which was assumed to have a uniform composition as follows: CO_2, 0.04 per cent; O_2, 20.93 per cent; and N, 79.03 per cent. In one or two of the early experiments the subjects breathed room air, in which case the composition of the air was determined by analyses of samples taken at the time the determinations were made.

During the course of a determination the subject lay motionless upon a cot bed. In the intervals between successive determinations he was allowed to move about on the bed and to talk. In this way subsequent restlessness, due to too long a period of enforced quietude, was avoided.

Results. In all, eleven experiments on ten different subjects were satisfactorily carried out. The results obtained are given on page 275.

Discussion. In ten out of eleven experiments an increase in oxygen consumption was observed after the oral administration of adrenalin chloride. The average increase of calculated heat production, including the single negative experiment, was 6.9 per cent. The most reasonable way of accounting for this increase is by the assumption that a certain amount of the adrenalin taken into the stomach was absorbed into the blood stream and, by means of its catalytic properties, increased the rate of oxidation within the body. The only possible alternative to this explanation is that the adrenalin stimulated activity of the smooth muscle of the stomach, and the increased metabolic rate was due to this increased activity. The work of Danielopolu and Carniol (6) showing that adrenalin taken by mouth caused an increase in gastric contraction has already been noted. Furthermore, the subjective effects reported by some of the subjects in these experiments, which are discussed below, point to a probable increase in activity of the musculature of the stomach. It is therefore quite possible that this increased muscular activity ac-

counts for a part of the increase in metabolic rate noted. However, it seems altogether unreasonable to assume that increases such as those noted in experiments 3, 4 and 6, ranging as they do from 10 to 15 per cent, could be brought about by increased activity of the stomach alone. Snyder (11) has shown that the contraction of smooth muscle is accompanied by the production of little or no heat, and it would seem safe, therefore, to assume that relatively little oxygen is consumed in this way. It would, therefore, appear that the part played by the stomach in bringing about the increase in metabolic rate was relatively small and unimportant. These experiments are, consequently, believed to constitute direct evidence that adrenalin chloride is to some extent absorbed from the stomach.

Boothby and Sandiford (12) have calculated that from the subcutaneous injection of 1.0 mgm. of adrenalin 50 calories of heat are produced in

TABLE 1

EXPERIMENT NUMBER	TOTAL CALORIES PRODUCED BY ADRENALIN	ADRENALIN ABSORBED	ADRENALIN TAKEN	PER CENT ABSORBED
		mgm.	*mgm.*	
1	6.65	0.133	6	2.2
3	9.85	0.197	5	3.9
4	8.25	0.165	10	1.7
5	3.01	0.060	8	0.7
6	6.61	0.132	10	1.3
7	4.85	0.097	7	1.4
8	4.25	0.085	5	1.7
9	2.45	0.049	5	1.0
10	2.02	0.040	3	1.3
11	6.23	0.125	5	2.5

excess of what would have been produced had not the adrenalin been given. This makes it possible to compute in a rough way the quantity of adrenalin absorbed in these experiments. In experiment 11 the effect of the adrenalin was only about half as great at the end of an hour and twenty minutes as it was forty-five minutes after administration. It would seem safe to assume, therefore, that by the end of two hours the effect would be entirely gone. In this experiment the largest increase in total calories per hour amounted to 6.2 calories. The average increase for the two hours during which the adrenalin was effective would be about half of this figure, and we may, therefore, take 6.2 calories as the heat produced in excess of what would have been produced had not the adrenalin been given. According to Boothby and Sandiford's figures, 0.124 mgm. of adrenalin would produce 6.2 calories of heat, and we may assume that this is approximately the amount that was absorbed in this

PROTOCOLS

SUBJECT	AGE IN YEARS	HEIGHT IN CENTIMETERS	WEIGHT IN KILOS	NORMAL					PLACEBO					MILLIGRAMS OF ADRENALIN GIVEN	FIRST DETERMINATION AFTER ADRENALIN					SECOND DETERMINATION AFTER ADRENALIN				
				Liters of air breathed per minute	Respiratory quotient	Total calories produced per hour	Calories per hour per square meter body surface	Metabolic rate (DuBois standard)	Liters of air breathed per minute	Respiratory quotient	Total calories produced per hour	Calories per hour per square meter body surface	Metabolic rate (DuBois standard)		Liters of air breathed per minute	Respiratory quotient	Total calories produced per hour	Calories per hour per square meter body surface	Metabolic rate (DuBois standard)	Per cent increase in heat production after adrenalin	Liters of air breathed per minute	Respiratory quotient	Total calories produced per hour	Calories per hour per square meter body surface	Metabolic rate (DuBois standard)	Per cent increase in heat production after adrenalin
F. H.	34	172.5	93.5	6.341	0.86	79.39	38.50	−2.5%						6.330	0.86	83.51	40.54	+2.5%	5.2%						
E. S.	24	173.0	70.3	5.497	0.80	76.15	41.39	+4.8%	Placebo omitted					5.086	0.78	70.21	38.15	−3.4%	−3.6%						
W. T.	31	181.5	96.0	6.669	0.89	81.00	37.33	−5.6%	5.332	0.81	72.85	39.59	+0.2%	5.077	0.86	90.06	41.50	+5.1%	12.0%						
M. G.	29	168.0	56.0	4.967	0.84	56.38	33.96	−14.0%	6.532	0.90	80.21	36.96	−6.4%	5.231	0.83	61.71	37.20	−5.8%	15.4%						
V. F.	24	172.0	66.5	Normal omitted					4.400	0.77	53.48	32.20	−18.5%	10						*Subject asleep*					
									5.188	0.75	63.59	35.90	−9.0%	8	5.449	0.74	66.60	37.63	−4.7%	4.7%	4.914	0.82	59.63	33.70	−14.4%	−6.2%
E. McE.	22	169.5	59.0	Normal omitted					4.731	0.803	61.49	36.60	−7.3%	10	4.928	0.790	68.10	40.50	+2.5%	10.7%	*Subject asleep*					
E. H.	22	180.0	69.8	5.027	0.761	72.40	38.51	−2.5%	5.041	0.805	68.21	36.33	−8.1%	7	5.309	0.710	73.06	38.86	−1.6%	6.6%	4.196	0.756	63.68	37.90	−4.1%	3.6%
C. A.	19	174.0	63.0	4.757	0.857	61.32	34.83	−15.0%	4.649	0.877	61.48	34.93	−14.8%	5	4.724	0.832	65.73	37.35	−8.9%	6.9%						
G. W.	25	175.5	64.1	Normal omitted					3.829	0.811	59.20	34.02	−13.9%	5	3.751	0.743	61.65	35.43	−10.3%	4.1%	4.092	0.808	59.51	34.20	−13.4%	0.5%
W. K.	21	168.0	60.3	5.202	0.754	69.33	41.27	+4.3%	4.926	0.738	69.27	41.23	+4.3%	3	5.319	0.760	71.29	42.43	+7.4%	2.9%	5.663	0.797	69.99	41.66	+5.5%	1.0%
F. H.	34	172.5	93.5	Normal omitted					5.643	0.771	76.45	37.11	−6.0%	5	5.883	0.756	82.68	40.14	+1.6%	8.1%	5.588	0.743	82.64	40.12	+1.6%	8.1%
																				Third determination after adrenalin					
																					5.423	0.785	79.51	38.61	−2.3%	4.0%

experiment. In a similar manner the amount of adrenalin absorbed in each of the ten experiments in which positive results were obtained has been calculated. In each case the assumption has been made that adrenalin was effective through a two-hour period and that one-half of the maximum increase in heat production observed in the experiment was the average increase in heat production for the two hour period. The figures obtained in this way are tabulated on page 274.

While the percentage of the adrenalin absorbed given in the above table is little more than a rough approximation, still the figures probably do indicate in a general way the degree of absorption that took place. The table also indicates that there is a high degree of variability in the percentage of adrenalin absorbed by different individuals.

In experiments 7 and 9 and in one other experiment not reported severe gastric disturbances followed administration of the adrenalin. The subjects in experiments 7 and 9 both complained of severe and continuous pain in the stomach, and vomiting occurred in both cases about ten minutes after the ingestion of the drug. Relief from the pain followed the vomiting, and when the determinations of the metabolic rate were made the subjects stated that they had no pain and felt normal in every way. By far the most severe gastric disturbance observed was that experienced by M. O. L. This subject was given 15 cc. of the 1:1000 solution of adrenalin. In about five minutes he was seized by pain in the stomach so severe and continuous that it was necessary to remove the face mask. This pain continued for approximately two hours. Finally a hypodermic injection of atropin was given, and within ten or fifteen minutes the pain was entirely gone. The subject vomited frequently during the first hour of the gastric disturbance and on several occasions the vomitus appeared bloody. In experiments 2 and 6 the subjects complained of pain in the stomach, which, however, lasted only a few minutes and was not severe enough to make it necessary to remove the face mask. It is possible that in these cases the pain may have been a factor in increasing the metabolic rate, although this seems unlikely since in each case the subject indicated that the pain had entirely ceased before the test was begun. Furthermore, the fact that the only subject who gave a negative result suffered from a pain in the stomach, presumably as a result of ingested adrenalin, would apparently indicate that the pain was not sufficiently severe to cause an appreciable rise in the metabolic rate. However, granting that the results obtained in the four cases in which there were gastric disturbances are questionable, there are still seven experiments in which there was no gastric disturbance and in every one of which there was a rise in the metabolic rate after the oral administration of adrenalin, the average rise being 8.2 per cent, which is 1.3 per cent higher than the average for all eleven experiments. In other words, the inclusion of the cases in

which gastric disturbances were suffered, decreases rather than raises the average increase in metabolism. The probable explanation of this lies in the fact that in the two cases in which vomiting occurred, very little adrenalin remained in the stomach.

Judging from the experiments reported here, it would appear that it is unsafe to give doses by mouth of 5 mgm. (5 cc. of the 1:1000 solution) or larger unless the tolerance of the patient is known. On the other hand, since these experiments indicate that the amount of adrenalin absorbed from the stomach is of the order of 1.7 per cent of the amount ingested, doses smaller than 1.0 mgm. would probably have little or no effect.

It should be noted that in both experiments 5 and 6 the subject was asleep during the second test made after the administration of adrenalin. As would be expected, both subjects showed a decided drop in the metabolic rate with the oncoming of sleep. This drop is about 10 per cent in experiment 5 and a little more than 6 per cent in experiment 6. It is of interest to note, however, that the rate of the subject during sleep after the administration of adrenalin was, in experiment 5, less than 5 per cent below the normal rate obtained before adrenalin was given, and in experiment 6 it was 0.9 per cent above the normal rate. According to Benedict and Carpenter (13), the metabolic rate is from 8 to 10 per cent higher when the subject is lying awake than when he is asleep. Therefore we may assume that in experiment 5 the metabolic rate in the second test was from 3 to 5 per cent above the normal, while in the second test of experiment 6 it was from 9 to 11 per cent above normal.

It is worthy of note that in all but two cases the basal metabolic rate obtained in these experiments was below the DuBois standard. This is probably due to the fact that all the subjects were either instructors or well-trained students. They were all familiar in a general way with the problem being investigated and were fully disposed to coöperate in obtaining reliable results. It was, therefore, to be expected that a greater degree of relaxation would be obtained than in subjects of no scientific training. In five of the experiments two determinations were made before the adrenalin was given. In three of these cases the rate obtained by the second determination was appreciably lower than that obtained by the first. In one case the second determination showed a slight rise in the metabolic rate and in the remaining case the second determination gave a rate practically the same as that obtained by the first determination. It would seem, therefore, that in a majority of the experiments the tendency was for the metabolic rate to drop during the course of the experiment. This assumption is further substantiated by the fact already noted that two subjects fell asleep toward the end of their respective experiments, while several others stated that they had difficulty in re-

maining awake. This makes the rise obtained after oral administration of adrenalin even more significant than it appears to be from the results obtained.

SUMMARY

In ten out of eleven experiments on adult men the oral administration of adrenalin chloride, in doses of from 3 to 10 mgm., was followed by a rise in the metabolic rate. The average increase was 6.9 per cent. It is believed that this rise was caused by the absorption of adrenalin from the stomach and probably the upper intestine and the extent of the rise indicates that the amount absorbed was of the order of 1.7 per cent of the quantity ingested although wide variations between different individuals were noted. Doses of 5 mgm. or more in some subjects cause severe gastric disturbances.

I wish to acknowledge my indebtedness to Prof. R. G. Hoskins for various helpful suggestions. I am also indebted to Mr. M. O. Lee for assistance in carrying out these experiments, and to various instructors and students who, at a cost of considerable time and inconvenience, served as subjects.

BIBLIOGRAPHY

(1) HATCHER AND WILBERT: The pharmacology of useful drugs. Chicago, 1915.
(2) BASTEDO: Materia medica, pharmacology and therapeutics. 2nd ed. Philadelphia, 1919.
(3) FUNK: Potter's Therapeutics, materia medica and pharmacology. 13th ed. Philadelphia, 1917.
(4) ROWNTREE: Med. Clin. N. Amer., 1923, vii, 177.
(5) TRIAS AND DORLENCOURT: Compt. rend. Soc. de biol., 1922, lxxxvii, 1189.
(6) DANIELOPOLU AND CARNIOL: Compt. rend. Soc. de biol., 1922, lxxxvii, 716.
(7) VINCENT: Internal secretions and the ductless glands. 2nd ed. London, 1922.
(8) BOOTHBY AND SANDIFORD: This Journal, 1923, lxvi, 93.
(9) BOOTHBY AND SANDIFORD: A laboratory manual on the technic of basal metabolic rate determination. Philadelphia, 1920.
(10) HENDERSON: Journ. Biol. Chem., 1918, xxxiii, 31.
(11) SNYDER: This Journal, 1916, xl, 138.
(12) BOOTHBY AND SANDIFORD: Physiol. Reviews, 1924, iv, 69.
(13) BENEDICT AND CARPENTER: Carnegie Inst., Washington, Publication no. 126, 1910.

STUDIES ON THE LOCOMOTION OF LEUCOCYTES

III. The Rate of Locomotion of Human Lymphocytes in Vitro

MORTON McCUTCHEON

From the McManes Laboratory of Pathology, University of Pennsylvania Medical School

Received for publication April 8, 1924

That lymphocytes are capable of amoeboid movement has been generally accepted for some years, and was recently confirmed by Sabin (1) in the case of normal human lymphocytes from the blood. The earlier writers, however, either explicitly state or imply that locomotion is shown by some, only, of the cells, and that such locomotion is extremely slow (2). Opposed to this view is the more recent work of Lewis and Webster (3), who studied lymphocytes in explants of human lymph nodes, and reported comparatively rapid rates of locomotion which, at their maximum, approach those of polymorphonuclear leucocytes.

It seemed desirable, therefore, in view of these contradictory reports, to study the locomotion of blood lymphocytes quantitatively, to determine what proportion of the cells are motile, how fast they move, and whether their motion is maintained in vitro over a number of hours. As the result of these studies it was found that the prevalent opinion, that lymphocytes are capable of only slight motility, is apparently erroneous, and that the reports of earlier writers to the effect that only a certain percentage of lymphocytes move at all were probably due, aside from faults of technic, to too short periods of observation.

Technic. The method employed for determining the velocity of leucocytes in vitro has been fully described in previous publications (4), (5). A drop of blood from the finger is allowed to spread between slide and coverslip, and is examined with the microscope in a warm box. The temperature is maintained at 37°C. The distance which each cell travels in five minutes is determined as previously described. Ten cells are examined and their average rate of locomotion is calculated in micra per minute. In the present study the lenses employed were an oil immersion (1.8 mm.) (except in the experiment with a quartz coverslip when a 4 mm. objective was used) and a drawing ocular. Observations were begun ten minutes after the preparation was placed in the warm box.

The following criteria were applied for deciding whether a cell is a lymphocyte. The size of the cell is nearly that of a red blood cell or slightly larger, about 8 to 11 micra in diameter, when measured in the living state.

Large lymphocytes were not included in this study, on account of the difficulty in distinguishing them from monocytes without the use of vital staining. The nucleus of the lymphocyte makes up the bulk of the cell, and has no permanent lobing; it lies in the front part of a moving cell. Cells containing specific granules were rejected, irrespective of their size.

All experiments were made with the blood of a single, supposedly healthy man.

Results. When lymphocytes are examined during the first hour after the preparation is made, only 54 per cent of the cells show *obvious* locomotion (the standard for obvious locomotion was arbitrarily taken as a distance of 10 micra—the average diameter of a cell—or more, in five minutes). But by the third hour the percentage of moving cells has increased to 68, and it continues increasing until, at the ninth hour, all the cells are in motion (see table 1).

TABLE 1

The percentage of lymphocytes in motion from the first to the eleventh hour in vitro, together with their average and maximum rate of locomotion

Each figure is based on from 30 to 50 cells observed during five experiments

HOUR	CELLS IN MOTION*	AVERAGE RATE PER MINUTE	MAXIMUM RATE PER MINUTE
	per cent	*micra*	*micra*
1	54	4	16
3	68	7	22
5	88	11	30
7	97	13	29
9	100	15	28
11	87	14	25

* Based on the number of cells moving 10 micra (= diameter of lymphocyte) or more in 5 minutes.

A corresponding change is found in the rate of locomotion. During the first hour this averages only 4 micra per minute,[1] but during the succeeding hours the cells move steadily faster until, at the ninth hour, the average is 15 micra per minute. Few observations were made beyond the eleventh hour; these indicated no marked change in rate for a number of hours. The maximal rate for any lymphocyte examined was 30 micra per minute, which is exactly the maximal rate given by Lewis and Webster (3), and approaches the speed of a polymorphonuclear leucocyte.

As reported in a previous publication (4), the average rate of locomotion for neutrophilic leucocytes of the same individual was 34 micra per minute, and the maximal rate was 47. These figures are based on observations

[1] Averages are based on resting as well as on moving cells.

made from a half to one and a half hours after the preparation was mounted and brought to a temperature of 37°C.

It seemed desirable to find out whether neutrophilic leucocytes show a similar acceleration with time. Observations were therefore made on these cells obtained from the same individual and examined under identical conditions. It was found that neutrophiles attain maximal velocity by the third hour (perhaps earlier); thereafter the rate of locomotion gradually lessens. A representative experiment is recorded in table 2. Unlike lymphocytes, all these cells display active locomotion even during the first hour, except a few which are obviously dead or dying, or which have adhered to chance foreign bodies.

Another point of difference is that these non-motile neutrophiles (they have not been included in the figures given in table 2) gradually increase in number as the experiment proceeds, whereas in the case of lymphocytes, no apparent decrease in the number of living and active cells occurs during

TABLE 2

The rate of locomotion of neutrophilic polymorphonuclear leucocytes from the first to the eleventh hour in vitro. A representative experiment

Each figure is based on 10 cells

HOUR	AVERAGE RATE PER MINUTE	MAXIMUM RATE PER MINUTE
	micra	*micra*
1	29	40
3	36	45
5	29	42
7	31	42
9	21	36

the period of observation. And as practically all lymphocytes are motile by the ninth hour, it would appear that they have undergone no deterioration.

No explanation can be offered at the present time of the peculiar behavior of lymphocytes in vitro described above. It was thought worth while, however, to determine whether alkali which might be given off by the slide and coverslip is a possible factor. For this purpose experiments were made using 1, glassware that had been boiled a number of hours and then soaked over night in acid alcohol; 2, glassware that had not been so treated; 3, quartz ware which, when covered with distilled water and incubated at 37° for a number of hours, produced no demonstrable change in the pH of the distilled water. Under all three conditions, the velocity curve was found to be practically identical. It is therefore unlikely that alkali from the glassware was an important factor in the results obtained.

It may be concluded from these experiments that practically all the

lymphocytes of the type described are capable of locomotion in vitro, often of relatively rapid locomotion. Contrary findings of earlier workers were probably based on too brief observations, or resulted from the use of unfavorable media or temperature conditions, though of course it is possible that lymphocytes from various individuals may behave somewhat differently. It should be noted that the observations of Lewis and Webster (3), which are in accord with those here reported, were made twenty-four hours or more after explanation of the tissue, so that sufficient time had elapsed for the cells to develop maximal motility.

If it is accepted that lymphocytes in vitro are capable of greater locomotion than has heretofore been supposed, then it would seem likely that in vivo also these cells are able to move actively through the tissues. Such a conclusion lends support to the view that lymphocytes reach inflammatory areas through active migration, though it does not disprove the possibilities of their being also passively carried by lymph currents or being formed by proliferation of cells already in the tissues (for discussion, see Marchand (6)).

SUMMARY

1. Of lymphocytes studied in vitro at body temperature 54 per cent showed obvious locomotion during the first hour. During subsequent observations more and more cells showed locomotion until by the ninth hour 100 per cent were in motion.

2. The average rate of locomotion increased from 4 micra per minute in the first hour to 15 micra per minute in the ninth. The maximal rate for a single cell was 30 micra per minute, approaching the speed of a neutrophilic leucocyte.

3. Neutrophilic leucocytes show by contrast only a brief period of acceleration, followed by a gradual slowing down.

4. The prevailing opinion that lymphocytes are capable of only extremely slow motion is erroneous, and is probably due to too brief periods of observation.

BIBLIOGRAPHY

(1) SABIN: Johns Hopkins Hosp. Bull., 1923, xxxiv, 277.
(2) RANVIER: Traité technique d'Histologie, Paris, 1875, 693.
WOLFF: Berl. klin. Wochenschr., 1901, xxxviii, 1291.
HIRSCHFELD: Ibid., 1901, xxxviii, 1019.
JOLLY: Arch. de Méd. Expér., 1903, xv, 54.
NEUMANN: Virchow's Arch., 1903, clxxiv, 41.
ASKANASY: Cent. f. allg. Path., 1905, xvi, 897.
(3) LEWIS AND WEBSTER: Journ. Exper. Med., 1921, xxxiii, 261.
(4) McCUTCHEON: This Journal, 1923, lxvi, 180.
(5) McCUTCHEON: Ibid., 185.
(6) MARCHAND: Verhandl, deutsch. Path. Gesell., 1913, xvi, 5.

ELECTRICAL RECORDS OF AFFERENT NERVE IMPULSES FROM MUSCULAR RECEPTORS

A. FORBES, C. J. CAMPBELL AND H. B. WILLIAMS

From the Laboratories of Physiology of the Harvard Medical School and the College of Physicians and Surgeons of Columbia University

Received for publication April 11, 1924

The existence of special afferent nerve impulses arising in muscles has been recognized for many years by physiologists. Histologically, structures known as muscle spindles of various sorts have been described in striated muscle. Similar structures are known to exist in tendons near the junction with muscle fibers. Sherrington (1) has shown that, in the so-called motor nerves supplying the limb muscles, from one-third to one-half of the fibers are afferent.

Sherrington and others have frequently called attention to the great physiological importance of impulses arising from these intramuscular receptors in the coördination of reflex and voluntary muscular activity. Abundant evidence has been accumulated indicating the influence of these proprioceptive impulses in regulating and modifying muscular action.

The condition of "decerebrate rigidity" has been demonstrated by Sherrington (2) to be "largely determined by centripetal impulses coming from the limb in question." Severance of dorsal roots abolishes the rigidity in those muscles in which arise the interrupted fibers. But the exact mechanism of this reflex rigidity and the nature of the stimuli which set up these afferent impulses are still obscure (cf. (3), (4), (5), (6)). Sherrington has shown that this decerebrate rigidity is the type of postural or "tonic" neural activity; therefore it is inferred that proprioceptive impulses play an important part in the maintenance of posture.

Additional proof of the existence of afferent impulses from muscle is found in the so-called "lengthening" and "shortening" reactions which Sherrington found in the condition of partial rigidity which he designates "plastic tonus," and in the "crossed reflex III" of Phillipson cited by Sherrington (4). In these experiments there can hardly be any doubt of the muscular origin of these nerve impulses, as the skin areas were rendered anesthetic, and the afferent twig supplying the only joint involved was sectioned. In "de-afferented" preparations all these phenomena are quite absent.

The knee jerk is now considered a reflex by the majority of investigators. The reasons mentioned by Sherrington (7) for doubt on the part of the older workers as to its reflex nature, were the great brevity of its latency and duration as compared with other reflexes familiar under the experimental conditions of that time. These reasons no longer exist, since Jolly (11) has shown that the time relations of the knee jerk, both as regards latency and duration, differed little from those of other reflexes studied under appropriate conditions (cf. also Forbes and Gregg (12), p. 143). Jolly's records also showed that the latency of the knee jerk was longer than it probably would be if due to direct mechanical stimulation. The knee jerk, then, seems to be a reflex which depends on proprioceptive impulses; that is, on stimulation of receptors within the muscle or tendon, stimulation presumably resulting from sudden tension.

The existence of afferent impulses arising from sensory endings in skeletal muscles is therefore well established. There are, however, several aspects of this phenomenon which remain obscure. So far as we are aware, the only published records of the action currents of these impulses are those of Jolly (11) incidental to the study of reflex time in the knee jerk of the cat, and those of De Meyer in the frog (17). Moreover, the nature of the stimuli which evoke them is not clearly understood. Sudden tension passively imposed, as in the case of the knee jerk, presumably constitutes an adequate stimulus. The nature of the stimulus in the sustained tension of decerebrate rigidity is, as already mentioned, more obscure. Also, it is not clear from the reflex effects just what stage in the shortening of a muscle due to its own contraction furnishes stimulation for the receptors.[1]

It is the purpose of the present communication to report experiments demonstrating the action currents of these proprioceptive impulses, resulting both from active contraction and from artificially applied tension, and, in the former case to present some data on the time of these impulses in relation to the contraction of the muscle which evokes them.

METHOD. Cats were used in all experiments, and in every case the nerve studied was the peroneal.

Two general types of stimuli were used. In the first, proprioceptive impulses were induced by active contraction of a muscle through stimulation of its motor nerve; in the second, by sudden tension applied to the passive muscle.

[1]Since this paper was sent to press there has appeared an important contribution to the subject of proprioceptive reflexes by Liddell and Sherrington (Proc. Roy. Soc., B., 1924, xcvi, 212), in which it is shown that, in the case of extensor muscles, tension, even while uniformly sustained, suffices to evoke reflex contraction in the stretched muscle.

Under chloroform-ether anesthesia a tracheal cannula was inserted, both carotids tied, and the animal was then decerebrated with the guillotine described by Miller and Sherrington (8) through the anterior half of the anterior colliculi. An incision was then made in the thigh (usually the right) exposing the sciatic nerve. The peroneal branch was dissected out all the way from the hip to its entrance into the tibialis anticus muscle, cut at the hip, and the proximal end of the distal portion led into a moist chamber, in which it was placed on a pair of leading-off electrodes of the Ag-AgCl type.

In the experiments with active contraction the nerve was stimulated by maximal break shocks. The femur and lower end of the tibia were rigidly clamped in a horizontal position, so that rotation of the ankle joint was in a horizontal plane. A thread was attached to the foot midway between the ankle and toes and fastened to a rigid support in such a way that the thread could be slacked or tightened at will, thus varying the amount of shortening which the muscle could attain before flexion of the ankle was stopped by the thread. Another thread was attached at the same point on the animal's leg and connected with a bell-crank lever of aluminum writing on a smoked drum. The lever in rising stretched a rubber band which served to minimize the overshoot due to inertia. A signal magnet marked the moment of stimulation and a Jacquet clock recorded time in fifths of a second. After finding that the latent period of the leg muscles was nearly constant in a series of experiments on different days, as long as the same apparatus was used, the signal magnet was omitted in the later experiments.

In order to be sure that galvanometric excursions correlated with muscular contraction were not due to mechanical disturbance of electrode contact, it was necessary to watch the nerve in the moist chamber and be sure that no such disturbance resulted from contraction. To provide a suitable control, it was necessary to establish a temporary block in the nerve between the contracting muscle and the leading-off electrodes. This was done in the earlier experiments with cold; in the later, with alcohol.

In the first group (with the cold block) a special moist chamber was used, arranged as in figure 1. The nerve at the entrance to the moist chamber nearest the muscle passed through a notch in the side of a glass tube through which a stream of cold brine could be passed at will. The stimulus was applied to the central end of the nerve, and both the resulting motor nerve impulses and the returning proprioceptive impulses were recorded at the leads lying between the point of stimulation and the muscle. Since the nerve was uninjured at both leads and throughout the intervening space between them, the result of this arrangement was that the action currents as recorded were all diphasic. This proved a

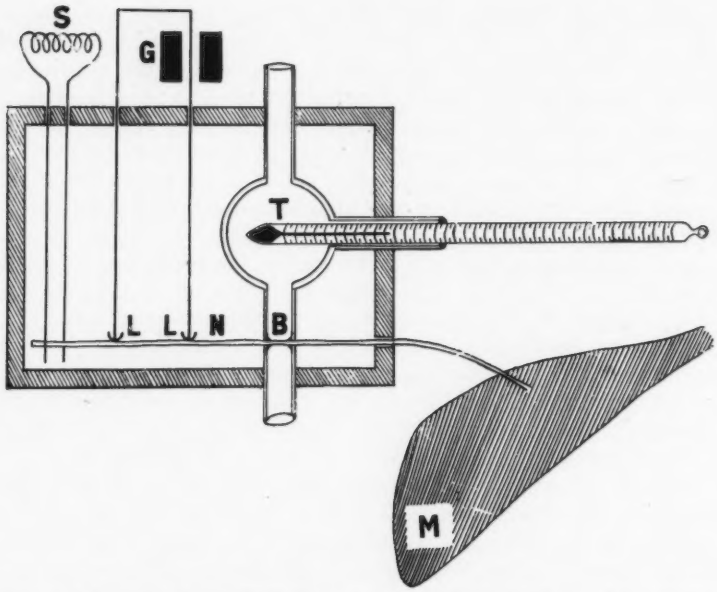


Fig. 1. Schematic diagram showing arrangement of apparatus for recording diphasic action currents of proprioceptive impulses, with cold block control.

S, secondary coil of stimulating inductorium; *G*, galvanometer; *N*, nerve; *M*, muscle; *LL*, leading-off electrodes; *B*, cold block; *T*, thermometer.

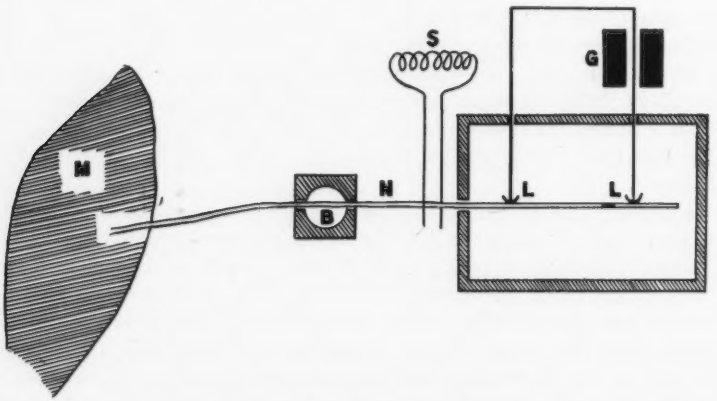


Fig. 2. Diagram of arrangement for recording monophasic action currents, with alcohol block control. Lettering as in figure 1, except *B*, which is alcohol narcotizing chamber.

considerable drawback for reasons which will presently appear. In the later experiments we therefore adopted an arrangement which enabled us to record monophasic action currents. To this end we rearranged the apparatus as shown in figure 2. We also found the cold block difficult to regulate, and for this reason replaced it, when rearranging the apparatus, with an alcohol block consisting of a slotted cup 8 mm. in diameter similar to that used by Olmsted and Warner (10), filled with Ringer solution containing 15 to 20 per cent alcohol.

In the second type of experiment, as already mentioned, the stimulus for the proprioceptive impulses was sudden tension applied by a device made for that purpose and christened for the sake of brevity a "leg-

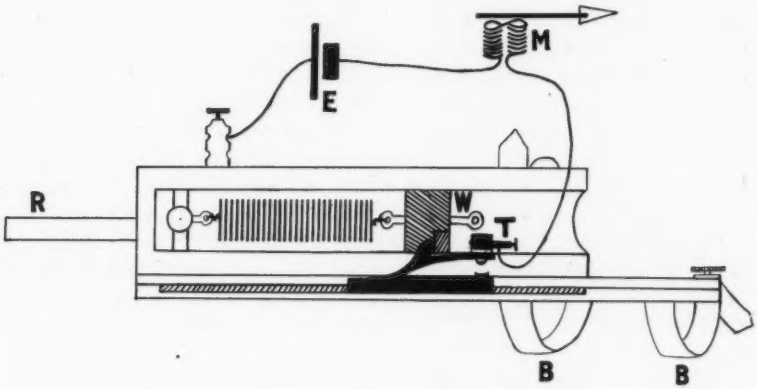


Fig. 3. Mechanical stimulating device (leg-puller)

W, weight to which tendon is tied; *S*, spring; *T*, trigger for releasing weight; *E*, cell providing current to operate signal; *M*, signal magnet; *B*, *B*, straps for binding apparatus to animal's foot; *R*, rod for clamping apparatus to rigid stand.

puller," electrical stimulation being used merely to determine whether or not the nerve was conducting. This mechanical device consists of a metal weight sliding in a groove and pulled by a strong spring when released by a pawl (fig. 3). A hole was cut in the skin over the internal malleolus, and the tendon of the tibialis anticus muscle was freed from the surrounding tissues, and cut. The leg-puller was securely strapped to the cat's foot and, by a clamp and stand, mounted on a small table separate from that upon which rested the cat and the moist chamber. The cut end of the tendon was securely lashed with strong thread, the free end of which was tied, leaving some slack, to the metal weight. The trigger operating the pawl which released the weight made an electrical contact which operated a signal magnet so placed that a shadow of the

armature fell upon the film of the recording camera, thus marking on the film the time of stimulation. The nerve innervating the muscle thus arranged was led into the moist chamber and laid on leading-off electrodes, as in the other type of experiment. Block in the course of the nerve was produced as already described, or in two instances by crushing the nerve between the muscle and the leads.

A few preliminary experiments were performed in the laboratory of the College of Physicians and Surgeons in New York, and the string galvanometer which has been in use in that laboratory for some years was employed. In these early experiments the diphasic method of recording, shown in figure 1, was used. They failed to show evidence of the proprioceptive impulses, probably because the tension of the galvanometer string was too great, for subsequent experiments have shown that with this arrangement, even with a slack string, without the aid of amplification the galvanometric excursions are at best very small. In all the later experiments a Hindle string galvanometer with 1.5 mm. air gap was used. In nearly all of these experiments a few records have been made directly with the galvanometer using a slack string, but a majority have been made with the aid of electron-tube amplification (9). The recording camera has been described in a previous paper (9, p. 418).

Observations. *Electrical stimulation.* In eighteen experiments, satisfactorily controlled by comparison of observations with and without nerve block, we have obtained electrical records clearly showing proprioceptive impulses. Six experiments with the arrangement of cold block as shown in figure 1, and one similar experiment using a galvanic block, showed these action currents of afferent impulses. Records from three typical experiments with the cold block method (fig. 1) are reproduced in figures 4 and 5. In figure 4 and in figure 5, columns A and C, are shown records made directly with the galvanometer; in columns B and D of figure 5 are records made in the same two experiments as those shown in columns A and C, but with electron-tube amplification. In the former are seen typical diphasic action currents representing the motor nerve impulses, followed, when the block was not applied, by small oscillations which suggest diphasic action currents whose first phase is in the direction opposite to that of the first phase in the motor impulse. This is what we should expect, since the afferent impulses traverse the leads in the opposite direction. In the amplified records the base line is not perfect; fine oscillations occur in absence of stimulation (9). How much these amount to may be seen by examining the part of the record before the application of the stimulus; larger excursions than these signify physiological activity. The initial excursion, marking the first phase of the motor nerve impulse, is so rapid that it fails to make a photographic record. After the disappearance of the

shadow it first reappears returning from the second phase. Its return is so rapid that the string overshoots and makes a complete double vibration before it comes to rest,—a result of the high string tension used in these records.

In both the direct and the amplified records it is clear that when there is no block excursions occur following the motor impulses at about the time when we should expect the afferent impulses to reach the leads. From the disappearance of these excursions when the block is established we may infer that they are due to action currents marking the return of proprioceptive impulses from the muscle. They might conceivably be due to shift of contact between nerve and leads from jog-

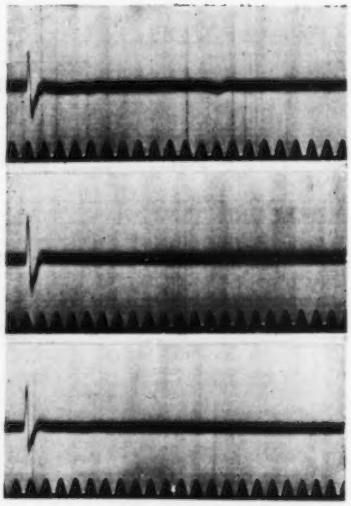


Fig. 4. String galvanometer records showing diphasic action currents in motor nerve in response to electrical stimulation, followed by action currents marking afferent impulses due to contraction of the muscle (Nov. 13, 1922).

Arrangement of apparatus as in figure 1. Hindle galvanometer; 4,800-ohm, gilded quartz string, diameter 2 μ, tension 100 m. per amp. (see Forbes and Ray (16)). Magnification 490.

Time shown by shadow of tuning fork; 1 double vibration = 0.01 second. Magnification and time record, where present, the same in all subsequent figures.

1, before nerve block. Temp. in tube, 22.2°C.

2, during nerve block. Temp. in tube, 7.2°C.

3, 12 minutes after block off. Temp. in tube, 18.0°C.

gling by the contraction of the muscle, but careful scrutiny satisfied us that no such motion occurred; moreover, the rapid character of the oscillations seems to preclude such an interpretation. Other evidence definitely bearing out this conclusion will be considered in the later experiments.

The excursions which follow those marking the action current of the motor nerve impulse, and which we have identified as of proprioceptive origin, are very minute when no amplification is used. Even when amplified they are small as compared with the response to direct stimulation of the motor nerve. Bearing in mind the all-or-nothing law of nerve response, there are two possible reasons for this difference. The first and more obvious is the small percentage of fibers involved in the

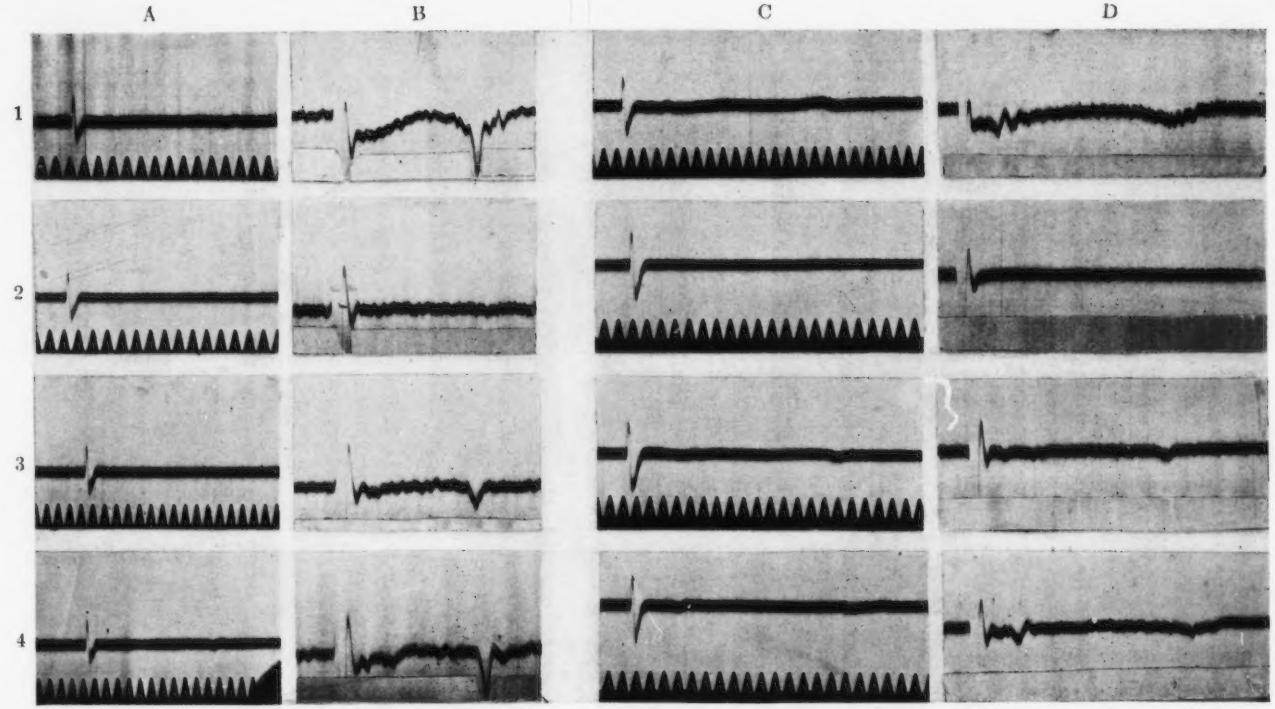


Fig. 5. Records illustrating the same points as figure 4 in two additional experiments. Same string used. A and C without, B and D with electron-tube amplification. Speed of film in all the same as in figure 4.

A, November 8, 1922. String tension, 100 m. per amp. (1), Before nerve block. Temp. in tube, 21°C. (2), During block. Temp., 6°C. (3), Partial block. Temp., 10°C. (4), 15 minutes after block off. Temp., 20°C.

B, November 8, 1922. Electron-tube amplification, 0.1 microfarad condenser damping; string tension 60 m. per amp. (1), Before block. Temp., 21.8°C. (2), During block. Temp., 8.5°C. (3), Partial block. Temp., 16°C. (4), 5 minutes after block off. Temp., 18°C.

C, December 12, 1922. String tension 100 m. per amp. (1), Before block. Temp. 23°C. (2), During block. Temp., 2°C. (3), Partial block. Temp., 7°C. (4) 13 minutes after block off. Temp., 16.8°C.

D, December 12, 1922. Electron-tube amplification; tension 58 m. per amp. (1), Before block. Temp., 19°C. (2), During block. Mercury too low to read. (3), Partial block. Temp., 12°C. (4), 13 minutes after block off. Temp., 16°C.

proprioceptive activity; not all of the peroneal nerve goes to muscles—there are some cutaneous branches—and in the muscular branches only half the fibers or less are afferent. This, however, in all probability does not entirely account for the small size of the observed action current and certainly does not account for its shape, which is not purely diphasic. The most probable explanation for this lies in the fact that the many individual impulses coming from the muscles are not synchronous. When the action currents are recorded diphasically a slight dispersion in time of the impulses in the individual fibers in a nerve trunk will suffice to throw the individual action currents far enough out of phase with each other to make the total observed effect almost disappear through interference. The second phase of the electrical change in a fiber coming synchronously or nearly synchronously with the first phase in a neighboring fiber would result in a neutralization of the electric response of the

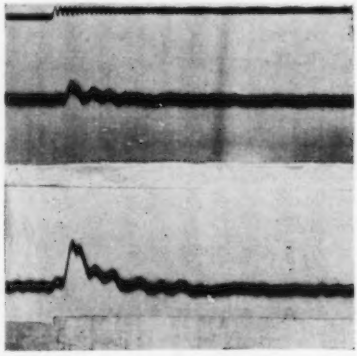


Fig. 6. Records showing responses of afferent nerve to mechanical stimulation of receptors. (March 1, 1923.) 17,000-ohm, gilded quartz string, diam. 2.75 μ, tension 208 m. per amp. Electron-tube amplification with 0.3 microfarad condenser damping. Speed of film 19 cm. per second.

(1), Diphasic response; (2), monophasic response. Nerve was crushed between leads after *1* and before *2;* No other change in set-up.

whole nerve (12, p. 149), (13). This would produce not a sharp, abrupt excursion of the string, but a smaller, more gradual excursion such as is seen in the records taken with the electrodes on uninjured nerve (figs. 4 and 5). Small oscillations such as appear in some records (e.g., fig. 5, D1) would be due to volleys of impulses from small groups of fibers responding approximately in unison.

It was to overcome this difficulty that we adopted the procedure illustrated in figure 2 which enabled us to damage the nerve between the electrodes and thus lead off monophasic action currents. The resulting record shows first the centripetal response of the entire nerve to direct stimulation, and then the response in the afferent fibers to the mechanical contraction of the muscle. The difference between diphasic and monophasic recording in the case of these proprioceptive impulses is best shown in figure 6 in which are reproduced records from an experiment of the

Mercury too low to read. (3), Partial block. Temp., 12°C. (4), 13 minutes after block off. Temp., 16°C.

second group, viz., one with direct mechanical stimulation with the leg-puller. In this case a good comparison was possible, since without disturbing or rearranging the apparatus, we could get monophasic records under conditions which were otherwise unchanged, by merely crushing the nerve between the leads after making the diphasic records. It will be seen in this figure that by rendering the responses monophasic, and thus eliminating interference from antagonistic phases in the component action currents, we have greatly increased the total excursion as recorded with the galvanometer. This is what we should expect on theoretical grounds, and gives support, if support is needed, to the view that nerve impulses are of essentially the same nature however evoked (cf. (12), p. 153).

Figure 7 shows typical records made with the monophasic method illustrated in figure 2. One of the most significant features of these records is that the galvanometric excursions are all in the direction which signifies a fall of potential at the electrode reached by the impulse. This is necessarily so if the excursions are due to action currents, but if they were due to some fortuitous source of current this fact would denote a remarkable coincidence. The amount of activity at any time is directly revealed by the excursion of the string, for with the monophasic method neutralization of electrical effect is eliminated. We can, therefore, much more easily appraise the results. In view of the all-or-nothing law of nerve response, the amount of activity means simply the number of fibers responding.

It is noteworthy that in almost every case the impulses which follow muscular contraction appear in two or more groups, scattered over a time varying from 20 to 160σ after the stimulus. We shall defer consideration of the significance of these groups till we have completed our account of the evidence which leads to the conclusion that these galvanometric excursions are indeed due to proprioceptive or afferent nerve impulses. It will be seen in figure 7 that all the excursions following that which resulted from direct stimulation disappeared when the alcohol block had become complete as shown by cessation of muscular contraction, and that they reappeared when the block was removed. In this respect they agree with the first group of experiments with the diphasic responses and the cold block.

Comparing the electrical records with mechanical records during the establishment of the block and during recovery when the block was removed, we find that proprioceptive impulses appear to be blocked more readily than the motor impulses, the electric responses in question being greatly reduced when the muscular contractions were but little affected. This was true in the case of both cold block and alcohol block, but was more clearly shown with the latter, and especially during recovery.

Mechanical stimulation. It is difficult to see how the galvanometric excursions which we have ascribed to afferent impulses could be otherwise explained in absence of any shift of electrode contact, especially as they disappear when their presumable source, muscular contraction, is prevented by nerve block, and since they undergo precisely the change we should expect when the nerve is damaged between the leads to make

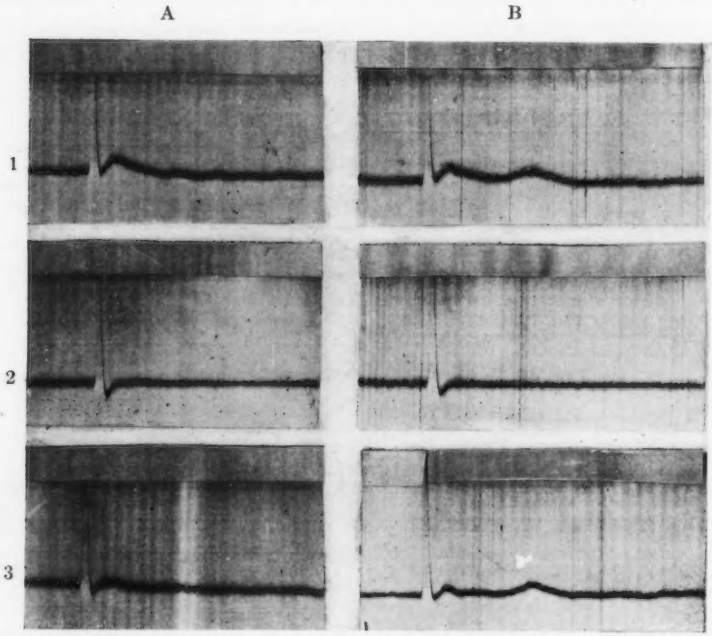


Fig. 7. Records of monophasic action currents in motor nerve in response to electrical stimulation followed by action currents of afferent impulses, showing disappearance of latter under nerve block.

Apparatus as in figure 2. Alcohol 15 to 20 per cent. 16,800-ohm string, tension 85 m. per amp. Electron-tube amplification with 0.2 microfarad condenser damping.

(1), before; (2), during; (3), after alcohol block.

A, October 4, 1923. Flexion of ankle limited.

B, October 10, 1923. Flexion of ankle not limited.

the action currents monophasic. Nevertheless it seemed desirable to reinforce this evidence by evoking the afferent impulses in a different way. Also, as mentioned in the introduction, it is not definitely established what part of the chain of events involved in muscular contraction constitutes the stimulus for the intramuscular receptors. For both of these

reasons we used as a stimulus sudden tension applied to the tendon by means of the leg-puller. Figure 6 shows a monophasic and a diphasic response evoked by this method and recorded with the aid of the amplifier; figure 8 shows monophasic records from two experiments. In some observations with this device we recorded the time of stimulation by the method already described. A control experiment showed that the actual

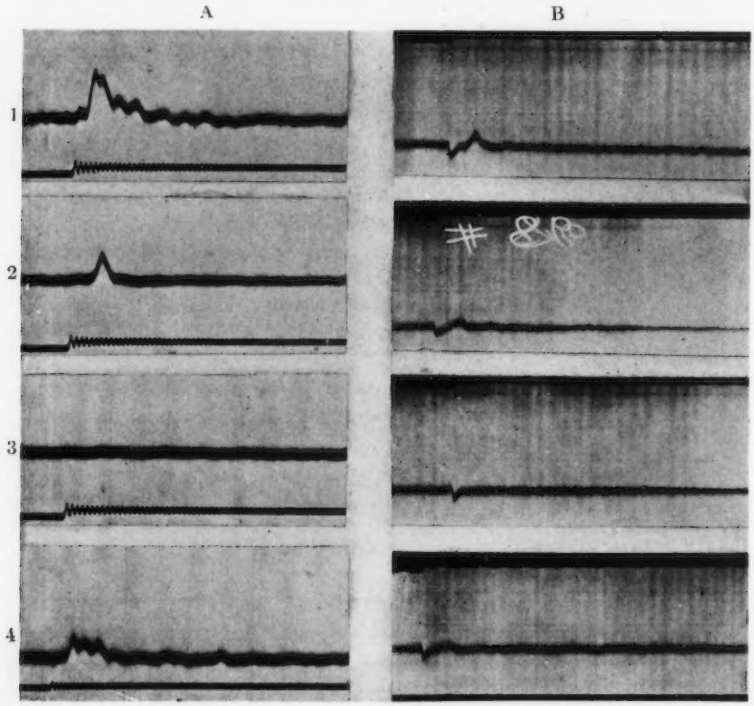


Fig. 8. Records showing disappearance under nerve block of afferent nerve impulses produced by mechanical stimulation of receptors.

A, March 1, 1923. 17,000-ohm string, tension 208 m. per amp. Electron-tube amplification. Signal magnet in trigger circuit. Cold block used. Speed of film 16 cm. per second.

(1), Before block. (2), Partial block. (3), Complete block. (4), 8 minutes after block.

B, Oct. 4, 1923. 16,800-ohm string, tension 85 m. per amp. Electron-tube amplification with 0.2 microfarad condenser damping. Signal magnet in trigger circuit. Nerve blocked with 18 per cent alcohol in Ringer solution. Speed of film 19 cm. per second.

(1), Before block. (2), Partial block. (3), Complete block. (4), 10 minutes after block.

pull upon the tendon occurred from 12σ to 18σ after the hammer was released. The electric response was found to begin about 13σ after the release of the hammer. The nearest lead was about 8 cm. from the muscle. Therefore the latency of the electric response was little if any greater than the conduction time in the nerve. The precautions to prevent joggling of the nerve in these experiments have already been mentioned, and careful scrutiny revealed no motion.

When the signal magnet was used there was generally an effect of its circuit on the galvanometer circuit that sufficed to cause an excursion of the galvanometer string, in the case of amplified currents. In the experiment from which figures 6 and 8A are taken, this excursion was only barely perceptible, but in others, e.g., figure 8B, it was about as large as that resulting from the action current. However, the excursion occurring when the key was closed was always in the opposite direction to that resulting from the action currents. For this reason, and also because the duration of the excursion due to electrical artefact was somewhat less than the latency of the physiological response, no confusion occurred from this cause. Moreover, this was controlled by occasionally taking records of the same experiment with the signal magnet disconnected and the electrical artefact thus eliminated, also by making occasional blank tests of the artefact by closing the signal magnet circuit, when connected with the animal, without applying tension to the tendon.

The records in figure 8 show the disappearance of the proprioceptive action currents upon the establishment of nerve block in two separate experiments. In one of these a cold block was used, in the other an alcohol block. In six experiments of this type the same result was obtained.

As in the case of stimulation through the motor nerve so in the present case the excursion in the galvanometer was always in the proper direction to signify action currents in the nerve. This fact, together with the regular disappearance of the response on establishing a block, seems to leave no doubt that the galvanometric excursions we are discussing were due to proprioceptive impulses arising in intramuscular receptors and traversing the afferent fibers in the nerve trunks.

As nearly as we can tell from the galvanometric excursion the duration of the action currents resulting from sudden tension applied in this way to the tendon is roughly from 20 to 50σ. Since at the temperature of the nerve in the moist chamber (approximately 20° to 25°C.) the duration of an individual action current at a given point in a fiber would not exceed 4 or 5σ at most (14), (15), there is clearly a considerable dispersion or overlapping in time of the impulses in the various fibers coming from the mechanically stimulated receptors, and possibly repetitive discharge as well.

The correlation of afferent impulses with the stages of muscular contraction. We have already mentioned the fact that the proprioceptive impulses in our records appear to traverse the nerve trunks in two or more groups separated by considerable intervals of time. We may take the first point of difference between the records obtained with and without nerve block as marking the arrival of the first impulses of intramuscular origin. In some experiments (fig. 5) excursions due to afferent nerve impulses are found as late as 145σ after the first impulses following the response of the nerve to direct stimulation. Usually there are two distinct groups of these impulses, one appearing soon after the response of the nerve to direct stimulation, the other at a time varying from 60 to 145σ later. The question arises as to what is the significance of these groups. They suggest that afferent impulses may be initiated at various stages of muscular contraction. For instance, the beginning of contraction might give rise to one group of impulses, and the end of the shortening process another. If this were the case we should expect a change in the recorded response upon alteration of the mechanical conditions; limitation of motion should give a different picture from that obtained with the limb free.

In order to test this point, we resorted in some of the experiments to the procedure already described whereby the motion of the foot was limited in varying degrees and at the same time recorded graphically. In this way we could determine the time occupied by contraction of the muscle, and then examine the electrical records to see whether the groups of action currents were so timed as to be clearly associated with the beginning and end of contraction. In the four experiments in which a signal magnet was used we found latencies, from stimulus to beginning of recorded contraction, varying from 12 to 35σ. In those experiments in which no signal magnet was used we assume a mean latency of about 20σ. The uncertainty here is not often apt to exceed 10σ, which is not serious, since the onset of the excursions we are considering is usually so gradual and often so irregular as to render precise measurements impossible in any case.

In figure 9 are records taken from the two experiments which were most completely controlled mechanically. The first two columns A and B, are from the same experiment (Oct. 4), A with the galvanometer connected directly with the nerve and with its string slack, to render it more sensitive, B with electron-tube amplification and with the string in the galvanometer tight; the third column, C, is from another experiment (Oct. 10). The records in the top row were made with the ankle free, i.e., flexion not restrained; those in the middle row were made with the foot tied to the support with only enough slack to allow partial flexion; those in the bottom row were made with the string tied tight, so limiting

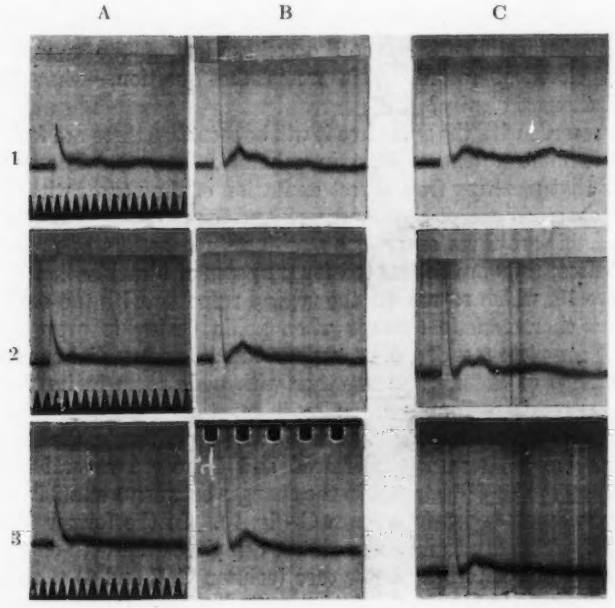


Fig. 9. Records showing the effect on proprioceptive impulses of varying the limitation of flexion in response to electric stimulation of motor nerve. (1), Little or no limitation; (2), medium limitation; (3), extreme limitation. Speed of film 16 cm. per second.

A. October 4, 1923. 16,800-ohm string, tension 340 m. per amp.

B. October 4, 1923. Same string as in A, but with electron-tube amplification, string tension 85 m. per amp., 0.2 microfarad condenser damping.

C. October 10, 1923. The same set-up as in B.

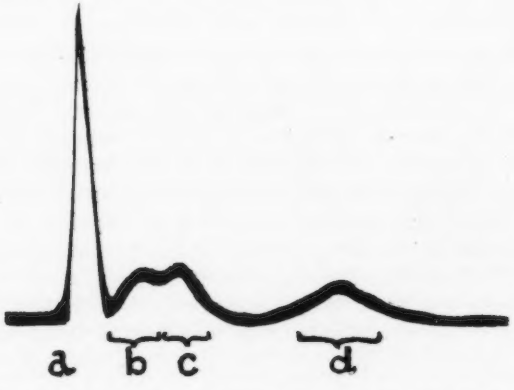


Fig. 10. Diagram of typical monophasic record for identification of characteristic excursions referred to in text.

the motion of the ankle that contraction was almost isometric. Obviously mechanical limitation of the contraction of the muscle does alter the electrical record. This raises the question,—what do the observed changes signify?

In the records with the ankle free it will be seen that there are four fairly distinct excursions (shown best in column A, no. 1). The first and largest is that resulting from direct excitation of the nerve; with this we are not concerned except as a landmark and for purposes of comparison. Following this are three excursions marking three groups of proprioceptive impulses; they are designated for purposes of identification *b*, *c* and *d* in figure 10, which represents the typical response with the ankle free. The most characteristic changes when ankle flexion is prevented are partial or complete merging of excursions *b* and *c* and partial or complete disappearance of excursion *d* together with a decrease in its duration. These changes were found with almost constant regularity in a large number of individual records in both of these experiments, the limitation of motion being alternately increased and decreased several times in each. They are best illustrated in the changes between 1 and 2 in column B and between 2 and 3 in column C (fig. 9). In the former case the merging of excursions *b* and *c* was almost complete even with only partial limitation of flexion; in the latter case (column C, 2 and 3) the merging was never complete even with maximum limitation. It should be noted that in the former experiment (Oct. 4) the whole sciatic nerve had been severed, and consequently there was no decerebrate rigidity in the antagonistic gastrocnemius muscle. In the latter experiment (Oct. 10)—as in all other experiments—the popliteal branch of the sciatic nerve was intact. Consequently there was not only some measure of decerebrate rigidity to oppose flexion, but also the possibility of a proprioceptive reflex (ankle jerk) in the gastrocnemius muscle, adding to the tension to which the tibialis anticus was subjected in consequence of its own contraction.

In considering the significance of our findings, the most reasonable supposition is that, as has already been intimated, tension is presumably the stimulus which excites the intramuscular receptors. Whether stimulation varies directly with tension, or rather with the rate of increase of tension, is a question. The dependence of the knee jerk upon sudden application of tension suggests *increase* of tension as the more important element. But the evidence for proprioceptive impulses in decerebrate rigidity, in which the tension is not increasing, but is sustained and constant, shows that the rate of increase is not the only factor.[2]

Let us see how the times of appearance of our electrical excursions can be correlated with times of either maximum tension or maximum rate of

[2]See also Liddell and Sherrington, Proc. Roy. Soc., B., 1924, xcvi, 212.

increase of tension. We have tabulated the measurements of time in six experiments in which simultaneous mechanical and electrical records were made, together with two others in which only electrical responses were recorded; the figures are shown in table 1. In the second column, following the date of the experiment, is the mechanical condition imposed on the muscle, as determined by the tightness of the thread which limited tension. In most of those marked "slack" the thread was not tied at all, and therefore there was no restraint to flexion. In those marked "taut," little motion was permitted. In those marked "medium," various amounts of slack were left, and flexion usually ended with an abrupt tightening of the thread. The third column shows the latency of mechanical response, when recorded. The fourth column shows the time from the stimulus to the height of contraction as indicated in the

TABLE 1

DATE OF EXPERIMENT	RESTRAINT OF ANKLE	LATENCY	TIME TO MAXIMUM CONTRACTION	a – b		a – d		LEADING OFF
				Beginning	Peak	Beginning	Peak	
		σ	σ	σ	σ	σ	σ	
Nov. 13, 1922	Thread taut			38	45	120	130	D
Nov. 16, 1922	Thread taut	24	90	30	35	82	87	D
Nov. 24, 1922	Thread slack	20	80	-12	19	43	50	D
Dec. 12, 1922	Thread taut	12	60	14	25	150	156	D
May 29, 1923	Thread medium			26	32	37	95	M
June 8, 1923	Thread medium	30	80	28	37	None		M
Oct. 4, 1923	Thread taut		52	19	25	78	82	
	Thread medium		48	20	27	82	90	M
	Thread slack		60	19	25	78	90	
Oct. 10, 1923	Thread taut		34	20	29	85	90	
	Thread medium		58	20	26	82	95	M
	Thread slack		60	19	26	84	110	

myogram (assuming a latency of 20σ when this was not measured). The next four columns show the times of what appear to be the most significant points in the electrical record after the beginning of the response to direct stimulation. This initial excursion from which we measure must have begun within less than 1σ after the stimulus was applied, and may therefore be taken as practically synchronous with stimulation. The first of these four columns (the fifth of the whole table) shows the beginning of the first excursion due to proprioceptive impulses; the next shows the time when this first excursion reaches its maximum (when excursions *b* and *c* (fig. 10) are distinct the peak of *b* is what appears in column 6). The next two columns show the beginning and the peak of the last well-defined excursion, *d*. All the figures appearing in this table are the averages from several records made under identical conditions

in each experiment. In the last column the arrangement of the leads is indicated; *D* signifies the diphasic arrangement (fig. 1), *M* signifies monophasic (fig. 2).

In drawing conclusions from this table we must remember that in the diphasic arrangement the impulses travel about 12 cm. from the stimulating electrodes to the muscle, and about half this distance in returning to the nearest lead; in the monophasic arrangement the stimulus is applied about 6 or 8 cm., and the nearest lead about 10 cm. from the muscle. The nerve is cooled nearly to room temperature for the greater part of its length. Therefore the time to be allowed for conduction of impulses, both motor and afferent, is roughly from 3 to 5σ. About half of this time is included in the latency of contraction, and the remainder should elapse between the beginning of contraction and the first appearance of proprioceptive impulses at the leads.

It will be seen that in general the first afferent impulses revealed in our records appear, according to the table, at about the proper time after the first recorded contraction. On November 24 the electric response preceding the first discernible contraction by 12σ, appears to be anomalous, but it is eminently probable that tension began to develop as long as this before the recording apparatus, with its considerable inertia, showed any motion. It may be significant that in this experiment the peak of this excursion corresponded closely with the first apparent mechanical contraction. In all the other experiments the corresponding excursion, *b*, appears to be closely synchronized with the first motion of the myograph lever, allowing 2 or 3σ for conduction time from the muscle. Considering the uncertain and probably variable delay in the appearance of contraction, due to inertia in the foot and lever, and considering the somewhat gradual onset of the galvanometer excursion, and the irregularity of the base line in the amplified records in which the excursions are most easily detected, the agreement is as good as we could expect. The records seem, then, to support the view that the first group of afferent impulses is initiated by the first onset of contractile tension.

It is not so easy to correlate the last group of afferent impulses shown in the records with a definite stage of contraction. We should naturally suppose that the end of contraction, especially when resistance is encountered through limitation of flexion, would be marked by a rather sudden increase in tension. We should look to this as the probable cause of the final group of afferent impulses appearing in the majority of experiments. A glance at the table shows apparent failure of correlation between the electrical excursion, *d*, and the mechanically recorded end of contraction. In some cases the peak of the electrical excursion precedes the mechanical peak by as much as 30σ, in others it occurs as late as 96σ after the mechanical peak.

But perhaps these figures do not militate as much as they appear to against the view that tension is the essential condition for exciting the receptors in question. In considering those cases in which the electric response appears before the recorded maximum of contraction, we should recall the imperfection of the recording device. The lever, even though of light construction and restrained in its motion by an elastic band, must have inertia enough to overshoot appreciably when moved by so sharp a pull as the maximal twitch of a large muscle, and the weight of the animal's foot must augment this overshoot considerably. It is therefore probable that the maximum tension which develops as the contracting muscle begins to encounter resistance, occurs some time before the muscle lever reaches its highest point. We believe that this may well explain the apparent discrepancy, and that this group of impulses may indeed mark a pronounced increase in tension as the contraction nears its limit.

Those other experiments in which the final electric response appeared longer after the height of contraction than could be explained by the conduction time of the nerve, present a different problem. Examining the mechanical records of December 12, in which this delay in the electric response was most marked, we find that after registering contraction, the muscle lever returned to a point below the original base line and then recorded another complete vibration on the drum; at the time when the final electric response appeared (about 150σ after the stimulus) the lever was about the middle of its second upstroke. Although we can see no clear cause of sudden tension at this particular moment, it is conceivable that the inertia of the foot and lever in their rebound may have caused the tension on the tendon to increase at that time. It is also possible that a secondary proprioceptive reflex in the gastrocnemius muscle (ankle jerk) may have imposed a sudden tension on the tibialis anticus muscle at that moment. This phenomenon might be the cause of the final electric response in most, if not all of our experiments, but against that view is the fact that in the experiment of October 4 the motor nerve to this muscle was cut, and yet an excursion corresponding approximately in time with those under discussion in the other experiments was regularly present in this one, although considerably smaller than in most (see fig. 9, B).

Although with our somewhat crude method of mechanical recording, the dependence of the final group of afferent impulses on tension is not clearly shown by the figures in the table, yet some such dependence is indicated by the changes in the electrical record when flexion is mechanically restrained. With partial limitation of motion, the foot gathers momentum, and then is stopped abruptly; with closer limitation, the tension is developed earlier, but since the foot is not allowed to gather

momentum it is not stopped with so sharp a jerk. In the latter case the electrical records, showing usually a merging of excursions *b* and *c* and an increase in the total of activity at this time, together with a decrease in the later excursion, *d*, are intelligible as indicating a shift of stimulation by tension to the earlier stages of contraction. Thus the change is in a general way qualitatively what we should expect, although the quantitative time relations as appearing in the table, do not wholly explain themselves.

Clearly our data do not suffice to discriminate between tension and rate of increase in tension as the more important element in the stimulation of the intramuscular receptors; neither do they serve to distinguish between the action of muscle spindles and tendon spindles. They do, however, all point to tension in some form as the source of excitation. The records made with active contraction of the muscle, evoked by motor nerve stimulation, are in harmony with this conception; and it is even more strongly supported by the experiments with mechanical stimulation, in which the afferent nerve response is definitely correlated with tension applied directly to the tendon.

SUMMARY

1. Afferent nerve impulses of intramuscular origin (known as proprioceptive impulses) have been shown by various investigators to play important parts in the coördination of muscular activity. They appear to be evoked by sudden tension, as in the knee jerk, and sometimes by sustained tension, as in decerebrate rigidity.

2. With a string galvanometer, usually aided by electron-tube amplification, we have recorded the action currents marking the passage of these proprioceptive impulses along the nerve trunk, using two distinct methods of evoking them,—active contraction of the muscle induced by stimulating its motor nerve, and tension artificially applied to the tendon.

3. In the differences between the records made with the diphasic and monophasic methods of leading off there is evidence which supports the view that the nerve impulse in the individual fiber is essentially the same, however evoked.

4. In the case of active contraction of the muscle in response to a single stimulus, the afferent impulses appear in two or three distinct groups. The first group appears to be initiated when the muscle begins to contract. The later groups probably depend on tension developed as the contracting muscle encounters resistance. As nearly as we could determine with the mechanical recording apparatus employed, the change in the electrical record of these impulses, resulting from limiting the motion of the contracting muscle, supported this view and pointed to tension as the essential element in the excitation of the intramuscular receptors.

We wish to thank Miss A. Hopkins for assistance in preparing the work for publication.

BIBLIOGRAPHY

(1) SHERRINGTON: Journ. Physiol., 1894, xvii, 211.
(2) SHERRINGTON: Journ. Physiol., 1897–1898, xxii, 319.
(3) SHERRINGTON: Brain, 1915, xxxviii, 191.
(4) SHERRINGTON: Proc. Roy. Soc., B., 1908, lxxx, 552.
(5) SHERRINGTON: Quart. Journ. Exper. Physiol., 1909, ii, 109.
(6) FORBES: Physiol. Rev., 1922, ii, 361.
(7) SHERRINGTON: Integrative action of the nervous system. New York, 1906, 86–87.
(8) MILLER AND SHERRINGTON: Quart. Journ. Exper. Physiol., 1915, ix, 147.
(9) FORBES AND THACHER: This Journal, 1920, iii, 409.
(10) OLMSTED AND WARNER: This Journal, 1922, lxi, 228.
(11) JOLLY: Quart. Journ. Exper. Physiol., 1911, iv, 67.
(12) FORBES AND GREGG: This Journal, 1915, xxxvii, 118.
(13) GASSER AND NEWCOMER: This Journal, 1921, lvii, 1.
(14) GASSER AND ERLANGER: This Journal, 1922, lxii, 496.
(15) ADRIAN: Journ. Physiol., 1921, lv, 193.
(16) FORBES AND RAY: This Journal, 1923, lxiv, 435.
(17) DE MEYER: Arch. internat. de Physiol., 1921, xvi, 64.

THE EFFECT OF DIFFERENT ANESTHETICS ON THE CATALASE CONTENT AND OXYGEN CONSUMPTION OF UNICELLULAR ORGANISMS

W. E. BURGE

From the Department of Physiology, University of Illinois

Received for publication April 15, 1924

The object of this investigation is to determine whether or not anesthetics produce a decrease in the catalase content and oxidative processes of unicellular organisms as is the case in animals such as the rabbit, dog, mouse and cat. The unicellular organisms used were Paramecium (caudatum) and Colpoda (sp. ?). The anesthetics were chloroform, ether, nitrous oxide and ethylene.

Anesthesia was produced by bubbling a mixture of oxygen and the anesthetic in the gaseous state through the liquid containing the organisms. Catalase determinations were made by adding 0.05 cc. of the organisms to 15 cc. of neutral hydrogen peroxide in a bottle and the amount of oxygen liberated in twenty minutes was taken as a measure of the catalase content. The oxygen consumption was determined according to the Rideal-Stewart modification of the Winkler method (1).

In figure 1 are given curves constructed from data of a typical experiment showing the effect of ether, chloroform, nitrous oxide, and ethylene on the catalase content of paramecia. It may be seen that chloroform decreased the catalase content 38 per cent during the two hours of anesthesia; ether decreased it 31 per cent; while nitrous oxide and ethylene had no effect. It should be mentioned in this connection that it was impossible to anesthetize paramecia with nitrous oxide and ethylene, while ether and chloroform produced a good anesthesia. It should also be mentioned that chloroform was more effective than ether in producing anesthesia. At the end of the two hours of anesthesia the organisms were removed from the liquid through which the anesthetics had been passed and transferred to fresh liquid. Immediately the organisms began to move around thus showing recovery from the effects of the anesthetic and in the chart it may be seen that at this time the catalase began to increase. It may also be seen that at the end of the six hours after anesthesia the catalase content of the organisms had risen well nigh to the original normal.

In the experiments in which the oxygen consumption of paramecia was determined 0.05 cc. of the organisms and 35 cc. of aerated water were used and the extent of the experiment was one hour. Chloroform decreased the oxygen consumption 44 per cent and ether 32 per cent while nitrous oxide and ethylene had no effect on the oxygen consumption. By comparing these results with those given in figure 1 it may be seen that

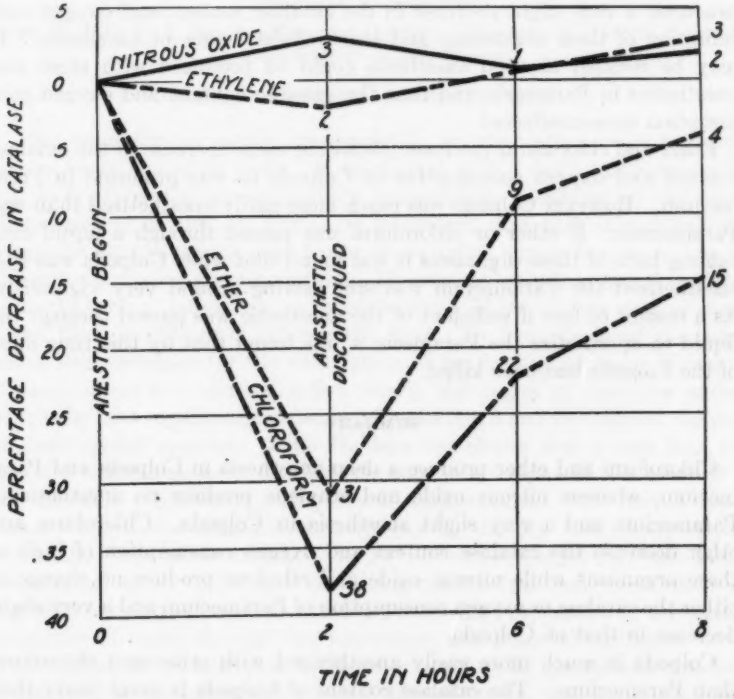


Fig. 1. Curves showing that ether and chloroform decrease the catalase content of paramecia, whereas nitrous oxide and ethylene do not, in keeping with the fact that chloroform and ether anesthetize the organisms while nitrous oxide and ethylene have no effect.

while nitrous oxide and ethylene had no effect on either the catalase content or the oxygen consumption, while ether and chloroform produced a decrease in both of these. Several experiments similar to the preceding were carried out with comparable results. In fact nitrous oxide as well as ethylene was bubbled through the liquid containing the paramecia for as much as six hours without producing any indication of anesthesia.

Colpoda was the organism used in the following experiments. These organisms were about half as rich in catalase as the Paramecia. Five hundredths cubic centimeters of Paramecia liberated approximately 70 cc. of oxygen from neutral hydrogen peroxide in twenty minutes while a similar quantity of Colpoda liberated around 35 cc. The anesthetics were passed through the liquid containing these organisms just as was done with Paramecia. It was found that nitrous oxide and ethylene produced a very slight decrease in the catalase content and oxygen consumption of these organisms, and also a slight degree of anesthesia. It may be recalled that no anesthesia could be produced with these two anesthetics in Paramecia and that the catalase content and oxygen consumption were unaffected.

Ether and chloroform produced about the same decrease in the catalase content and oxygen consumption of Colpoda as was produced in Paramecium. However Colpoda was much more easily anesthetized than was Paramecium. If ether or chloroform was passed through a liquid containing both of these organisms it was found that when Colpoda was well anesthetized the Paramecium was still moving around very vigorously. As a matter of fact if sufficient of the anesthetic was passed through the liquid to anesthetize the Paramecia it was found that by this time most of the Colpoda had been killed.

SUMMARY

Chloroform and ether produce a deep anesthesia in Colpoda and Paramecium, whereas nitrous oxide and ethylene produce no anesthesia in Paramecium and a very slight anesthesia in Colpoda. Chloroform and ether decrease the catalase content and oxygen consumption of both of these organisms while nitrous oxide and ethylene produce no change in either the catalase or oxygen consumption of Paramecium and a very slight decrease in that of Colpoda.

Colpoda is much more easily anesthetized with ether and chloroform than Paramecium. The catalase content of Colpoda is much lower than that of Paramecium.

BIBLIOGRAPHY

(1) Rideal-Stewart: Analyst, 1901, xxvi, 141.

THE PERIPHERAL WATER LOSS IN RABBITS AS A FACTOR IN HEAT REGULATION

ROBERT W. KEETON

From the Otho S. A. Sprague Memorial Institute, Laboratory of Clinical Research, Rush Medical College, Chicago

Received for publication April 18, 1924

Heat regulation in rabbits surrounded by an atmosphere whose temperature is above that of the body. If an animal with a body temperature of 102°F. is exposed to an atmospheric temperature (measured by dry bulb thermometer) of 104° to 110°F., it is evident that this animal will no longer be able to radiate heat to the surrounding atmosphere or to lose it through conduction and convection. His sole and only heat loss then must be in the form of latent heat of vaporization. Hunt (1) has studied man under these conditions of extreme dry heat. He has calculated the large water loss necessary for the elimination of the heat produced. Haldane (2) and others have defined rather clearly the degree of humidity under which the heat regulating mechanism breaks down and the clinical picture of heat stroke appears. Thus Haldane has shown that a man kept at rest in a still atmosphere, of which the wet bulb temperature exceeds 88°, will develop a gradually rising rectal temperature. This rise is continuous and is not influenced in any way by the dry bulb temperature of the air. The latter may be 3° or 10° above the critical wet bulb temperature of 88°F. The heat regulating mechanism then fails when a degree of humidity is reached which prevents the evaporation of adequate quantities of water through the respiratory and external surfaces.

The rabbit differs from man in the absence of sweat glands and thus he is deprived of an important avenue through which the water may be made available for evaporation. As is well known, the jack rabbit in his native habitat is found on the arid plains of Arizona and New Mexico and in other similiar places where the summer temperature frequently exceeds that of the animal. There may be some curtailment of the animal's physical activity during the periods of excessive heat but no one would suggest that the rabbit does not lead a highly successful life under such climatic conditions. It becomes of interest therefore to study this problem of heat loss in an animal devoid of sweat glands.

Figure 1 shows the apparatus used to reproduce the temperature conditions existing during the summer months in the animal's native habitat.

Many attempts were made to dry the air by passing it through calcium chloride and sulphuric acid towers. These attempts were uniformly unsuccessful. So the plan was adopted of drawing cold outside air into the box at the rate of 20 to 22 liters per minute. This air was relatively dry because the water had condensed out in the process of cooling. The electric lights shown in the figure were sufficient to rapidly raise the temperature of the incoming air to the desired level. Those days were selected for the experiments on which the temperature of the outside air was low enough to give the desired degree of dryness or humidity in the experimental chamber.

Table 1 gives the results of 16 such experiments conducted on one animal (rabbit VII). Confirmatory experiments were obtained on three other animals but it seemed unnecessary to include these data.

It is noted that an appreciable rise in the animal's rectal temperature occurs as he is exposed to air having a wet bulb temperature approximat-

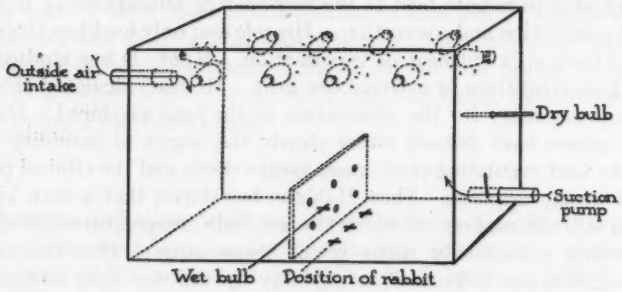


Fig. 1

ing 80°F. or more. This may be named as the critical wet bulb temperature for the rabbit's heat regulating mechanism. Below this point a rise of 0.6 to 1.0° may occur. However, it is well known to all experimenters, familiar with rabbit behavior, that such a rise is without significance. The animal's respirations may be increased and he may lie stretched out in the cage, but his general condition shows no evidence of discomfort. As soon as this critical point is passed the animal's appearance changes definitely. He becomes uneasy and may often change his position. The respirations increase rapidly and may reach a rate at which they are counted with difficulty. Drops of moisture appear about the muco-cutaneous junction of the nose and mouth, and the hair for 1 to 2 cm. around these regions becomes moist. This suggests the possibility that the partition of water from the buccal surface may be regarded as analogous in a sense to sweating, but more probably it perhaps represents condensation water which forms with slight cooling of the saturated

expired air. However, it is to be exphasized that this phenomenon does not occur until the rectal temperature begins to rise, and that it is not effective in preventing such a rise. Its appearance is simply coincident with the loss of temperature control. It may further be stated that the feet and flanks of the febrile animals have been palpated and that in no case was detectable moisture found. It would seem therefore that we have no experimental evidence that the rabbit utilizes a sweating mechanism in the control of his temperature.

Heat regulation in rabbits when peripheral heat loss is prevented. If therefore the rabbit can eliminate all of his heat in the form of latent

TABLE 1

Prevention of all heat loss with the exception of latent heat of vaporization, rabbit VII

DATE	DURATION OF EXPERIMENT	TEMPERATURE OF BOX		RABBIT'S TEMPERATURE		
		Wet	Dry	Start	Finish	Change
	hours					
12- 4-23	2	82	104	103.0	106.6	3.6
12- 5-23	2	82	104	103.0	105.6	2.6
12- 6-23	2	90	104	103.0	106.8	3.8
12-12-23	1½	83	104	103.0	105.0	2.0
12-13-23	1½	88	110	102.8	104.4	1.6
12-14-23	2	83.5	107.6	102.8	105.3	2.5
1-25-24	2	75-77	104-106.7	102.9	105.4	2.5
12- 7-23	2	82	104	103.0	104.2	1.2
12-17-23	2½	79	104	103.2	103.2	0.0
12-18-23	4	79	104-107.6	102.2	102.6	0.4
12-19-23	4	79	104-107.6	103.1	103.6	0.5
1- 5-24	2	66-68	102.2-105.8	103.4	104.0	0.6
1- 7-24	2	71-77	102.9-106.7	103.2	104.2	1.0
1-11-24	2	71-81	103.1-111.2	102.6	103.8	1.2
1-22-24	2¼	70-76	102.2-106.7	102.6	103.2	0.6
1-24-24	2¼	68-75	102.2-105.8	102.6	103.4	0.8

heat of vaporization and if we have no evidence of the existence of an adequate sweating mechanism, it is of great importance to know whether all of this water can be evaporated from the respiratory surface. Figure 2 shows the apparatus which was used in this study.

On one set of experiments a rabbit was used from which the ears had been removed three or four months previously. In the other series the ears of the animal were strapped close to the head with adhesive tape to reduce to a minimum the skin surface exposed to outside air. It was considered that the head covering was sufficient in these cases to render an amputation unnecessary. The calorimeter (fireless cooker) was lined with a felt cartridge as indicated in the cross section. The head of the fireless

cooker was well insulated as the cross section shows. However, there was an opportunity for the loss of heat by conduction through the edges of the head plate. To prevent any heat loss in this manner or through the covering of the face, the whole apparatus was placed in the electric lighted box. The latter could be maintained at any desired level. Outside of the apparatus an electric fan was so adjusted that a strong stream of cool fresh air could be blown against the animal's nose. This prevented any rebreathing. It is obvious that in such an experiment all the heat must necessarily be lost through the respiratory surface.

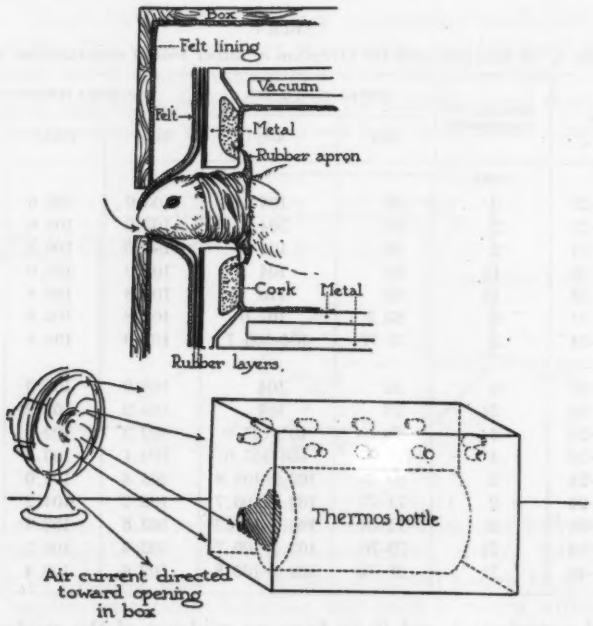


Fig. 2

Table 2 summarizes eight experiments on rabbit II, whose ears were previously amputated. The duration of the experiment was one and one-half hours. Since rabbits are almost continuous feeders and develop acidosis rather rapidly when fasted, the experiments were made on animals from whom food had been recently removed. It will also be noted that the temperature and the humidity of the inspired air were quite low so that these factors favored the ready loss of heat. Reference now to the table shows that within this short time the animal had begun to retain heat as shown by the rise in rectal temperature.

Table 3 summarizes the results of four experiments of longer duration on a normal healthy rabbit.

It is evident that with other factors constant the height of the temperature rise is dependent on the length of the experiment.

Table 4 shows the behavior of the same rabbit (VII) when the experiment was started with a higher initial level of the rectal temperature.

TABLE 2

Prevention of peripheral heat loss, healthy earless rabbit II

Ears removed several months previously

DATE	LAST FOOD	TEMPERATURE OF BOX	TEMPERATURE OF AIR			RABBIT'S TEMPERATURE		
			Wet	Dry	Humidity	Start	Finish	Change
					per cent			
Oct. 22	Food in cage	80.6	54	70	33	103.8	105.1	1.3
Oct. 23	12–18 hours	96.8	58	72	42	103.0	104.0	1.0
Oct. 24	Food in cage	104.0	50	66	29	103.4	105.8	2.4
Oct. 25	12–18 hours	96.8	52	58	66	103.2	105.0	1.8
Oct. 26	Food in cage	102.2	52	57	72	103.4	104.6	1.2
Oct. 29	Food in cage	104.0	60	75	40	103.0	105.2	2.2
Oct. 30	12–18 hours	104.0	59	78	30	103.2	104.8	1.6
Oct. 31	Food in cage	104.0	61	78	36	103.2	106.0	2.8

Duration of experiment, 1½ hours.

TABLE 3

Prevention of peripheral heat loss, normal rabbit VII

Normal healthy animal. Ears intact but strapped to head with adhesive tape so as to reduce surface.

DATE	DURATION OF EXPERIMENT	TEMPERATURE OF SURROUNDING BOX	TEMPERATURE OF RESPIRED AIR			RABBIT'S TEMPERATURE		
			Wet	Dry	Humidity	Start	Finish	Change
	hours				*per cent*			
Nov. 7	1¾	100.4	58	78	27	103.4	105.8	2.4
Nov. 8	1¾	104.0	57	74	33	103.8	105.8	2.0
Nov. 20	3½	105.8	59	73	35	103.0	109.0	6.0
Nov. 21	4½	105.8	56	74	29	103.6	111.5	7.9

Rabbit recovered well from all experiments.

Typhoid vaccine injections were used to establish these higher levels. The injections were given both intravenously and subcutaneously. When the desired higher temperature was reached and had maintained itself for a period, then the experiment was started. At such levels the heat production of course was greater so that the subsequent temperature rise was somewhat greater.

The experiments incorporated in tables 2, 3 and 4 show conclusively that when a rabbit is allowed to breathe relatively dry cool air, and that when all heat loss through the skin surfaces is prevented, the animal is unable to eliminate all his heat through the respiratory surface.

Is the respiratory surface adequate for total heat loss? In order to check the correctness of these experiments, the oxygen consumption and carbon dioxide production were determined in experiments which lasted from one to two hours. A glass bell jar was placed over the earless rabbit's head and clamped to the fireless cooker. Through this jar the air was circulated with a force pump. The outgoing lead from the bell jar was connected with a gasometer so that a sample of the circulating air could be collected when desired. Such samples were usually taken four times in two-hour experimental period, at intervals of thirty minutes. The first sample was collected fifteen minutes after the beginning of the

TABLE 4

Prevention of peripheral heat loss, vaccine rabbit VII

The vaccine was injected at varying intervals before rabbit was put into thermos bottle in order to get different temperature levels.

DATE	TYPHOID VACCINE		DURATION OF EXPERIMENT	TEMPERATURE OF SURROUNDING BOX	TEMPERATURE OF RESPIRED AIR			RABBIT'S TEMPERATURE		
	Dosage	Method of injection			Wet	Dry	Humidity	Start	Finish	Change
	millions		*hours*				*per cent*			
Nov. 9	2500	Intravenously	1¾	98.6	58	72	42	104.4	106.4	2.0
Nov. 12	1250	Intravenously	1¾	102.2	58	74	36	105.4	108.4	3.0
Nov. 14	2200	Intravenously	1¾	100.4	58	70	48	106.4	109.4	3.0
Nov. 19	2500	Intravenously	1¾	104.0	59	75	37	104.2	106.1	1.9

experiment and the last, fifteen minutes before its termination. A gas analysis[1] was then made on the composite sample. The analytical figures for oxygen were not entirely satisfactory because of the very slight difference between the values of the sample and outside air.

In this way it was found that the rabbit's oxygen consumption showed a wide range of variation. The lowest figure obtained corresponded to a heat production of 5.7 calories per hour, while the average of the higher values showed a production of 10 calories per hour. However, we are safe in accepting these approximate figures for the sake of computation, since in these experiments there was no appreciable rise in the rectal temperature of the animals. With a rising rectal temperature incident

[1] The author wishes to express his thanks to Miss Eva M. Russell of the Respiration Laboratory of the Presbyterian Hospital, who was kind enough to make the numerous gas analyses upon which the calculations below are based.

upon the accumulation within the animal of uneliminated heat there would be a corresponding rise in the oxygen consumption. If then this theoretically minimal quantity of heat can not be eliminated, the experimental findings will be substantiated. The question may now be more specifically stated. Can a rabbit lose through the respiratory surface 5.7 calories per hour? If he can lose 5.7 calories per hour, can he lose 10 calories per hour?

There are two methods by which heat may be lost from the respiratory surface. In the first place, it may be transferred by conduction to the cooler inspired air. This heat then is utilized in raising the air from the temperature at which it is inspired to the temperature at which it is expired (rabbit's body temperature). In the second place, it may be lost as latent heat of vaporization. In this case the amount lost would correspond to the difference in the quantity of moisture carried by the inspired and expired air.

The loss of heat by conduction may be considered first. One thousand grams of water (1 liter) raised through 1°C. requires 1 calorie. Since the specific gravity of air is 0.00129 then one liter of air weighs only 1.29 grams. Now the specific heat of air is 0.24 of that of water. Therefore 1 liter of air or 1.29 grams of air raised through 1° would require 0.00031 calorie. The temperature of the inspired air was rarely below 18°C. so that if this air is warmed to 40°, then the temperature of the air has been raised through 22°. One liter of air (1.29 grams) raised through 22° would require $0.00031 \times 22 = 0.0068$ calorie. In order to liberate one calorie, then, the rabbit would have to breathe 147 liters of air per hour. This therefore can not be regarded as a very important quantitative source of heat loss.

The loss of heat in the form of latent heat of vaporization may be considered next. At 40°C. one liter of water requires 574.2 calories for its vaporization. Hence then 5.7 calories will evaporate at 40° $\frac{5.7}{574.2} =$ 0.0099 liter of water, say for the sake of convenience 10 cc. The rabbit must therefore evaporate from 10 cc. to 20 cc. of water per hour in order to eliminate all of his heat as latent heat of vaporization. If it be assumed that the rabbit's expired air is saturated at approximately a temperature of 40°, then one liter of air under such conditions would contain 0.0507 gram of water. The rabbit must then breathe $\frac{10}{0.0507} = 197$ liters of air per hour to eliminate 10 cc. of water or 394 liters to eliminate 20 cc. of water.

The rabbit's ventilation has not been measured and since no very easy method for doing this is apparent, an estimation will be attempted. Man in the course of ordinary breathing ventilates himself with approximately

600 liters per hour. Some authorities place the value at half of this, 300 liters per hour. If we assume that a 2-kilo rabbit will require $\frac{1}{30}$ of this ventilation, he will then breathe 20 liters per hour. The rabbit's respirations are quite variable as to rate. They frequently attain the rate of 80 to 120 per minute, but of course it is reasonable to suppose that with such a rate the quantity of air inspired at each respiration is reduced. If we assume that the rabbit may increase his ventilation six times over that of the amount required for a man of similar body weight, he would still be able to respire only 120 liters per hour. Such a ventilation rate might enable him to eliminate 5.7 calories per hour, if it is assumed that the most favorable possible conditions of inspired air obtain (namely, an absolutely dry inspired air and an elevation of this air through 22°C.). It is obvious that such favorable experimental conditions cannot be uniformly reproduced experimentally. From these considerations it is to be concluded that the experimental results are correct and that it is impossible for the rabbit under any ordinary laboratory conditions to lose all of his heat from the respiratory surface. However, it was shown under the first set of experimental conditions that a rabbit could lose all of his heat as latent heat of vaporization. If this is possible and if the respiratory surface is insufficient to accommodate the traffic in water vapor then the periphery must eliminate appreciable quantities of water.

Mechanism of the peripheral water loss. The method of this water loss is of great interest. A careful search has been made of the literature for any studies showing the existence of sweat glands in the rabbit. None have been found As was noted in a previous section of the present paper, examination of the rabbits who have been exposed to high temperatures has not revealed any evidence of palpable or visible water, until the heat regulating mechanism has failed and the rectal temperature of the animal has begun to rise. At this time droplets of water appear on the hair at the muco-cutaneous junctions of the nose and mouth. Microscopical examination of the skin of this region has not revealed any sweat glands. It was suggested that this water might be due to a type of rudimentary buccal sweating which as is well known in dogs becomes an effective method of water loss. Or it may simply represent condensation water which appears with cooling of the saturated expired air. It is safe to conclude therefore that the rabbit has no adequate sweating mechanism and that the water loss in question must be partitioned in some other way. The ears of the rabbit are relatively devoid of hair. They are abundantly supplied with large blood vessels and are richly innervated with vasomotor nerves. If the cervical sympathetic nerve is cut the arterioles immediately fill up, numerous capillaries, which were formerly closed, become patent and the whole ear becomes distinctly warm to touch.

In short, the ear is composed of numerous organized colloidal masses, manifesting varying degrees of hydration, which the histologist calls "cells." Between these gel-like masses (cells) flow colloidal liquids of lesser viscosity (lymph and blood). Covering these structures there is a gel layer (the skin) which because of its relation to nutrient fluids and the air or because of other reasons, is distinctly less hydrated. Now each gel or sol develops its own vapor tension, and so, if the air to which these are exposed is changed continuously, there will be a continuous and measurable water loss. The molecules of water simply wander out of these colloidal systems, assume the vapor phase, and are removed by the air currents. This may be spoken of as water loss by direct vaporization or distillation. If vaso-dilatation occurs, then the proportionate quantity of protoplasm existing in the form of colloidal liquids and in a state of lessened viscosity is increased. This means that the surface from which evaporation may occur is proportionately increased. If now to this increase in evaporating surface is added a relatively unsaturated (dry) air, we have fulfilled two important conditions for vaporization.

This form of water loss should be sharply contrasted with that lost as sweat. Sweat is not partitioned directly through the skin layers. It is formed in the tubular glands and reaches the surface through the duct. The cells lining these tubules doubtless are in a state of greater hydration than the ordinary skin cells. Under proper conditions they may squeeze out of their surface films droplets of water which hold in solution a certain amount of salts and nitrogen compounds. This water existed in the colloidal system in a liquid phase and it still exists in the sweat in a liquid phase. There has been a transfer of water and other substances from a more to a less complex solution. Physiologically we call this process "secretion of sweat." If one seeks in a simple colloid system for an analogy, it may be found in the syneresis water developing in an agar slant culture tube. Now this water in the sweat, when exposed to the atmosphere, is evaporated. Such an evaporation should be spoken of as sweat vaporization. It is an indirect method of water loss and is to be contrasted with the direct tissue vaporization discussed above. If we need a biological analogy for the existence of this direct tissue vaporization we may find it in the leaves of plants where the process is highly active. If one were inclined to speculate further be might expect to find that the rabbit's ears are active in the gaseous exchange also.

Methods of water loss in man. The establishment of this form of water loss in animals devoid of sweat glands and the recognition of its importance in heat control raises the question of its existence and importance in man. In man water is lost in the form of sweat and insensible perspiration. Concerning the mechanism of the formation of the latter a bitter battle has been waged in the literature. One group of investigators has main-

tained that this water loss is always associated with activity of the sweat glands. The activity is regarded as either of a very low grade or intermittent in character. The other group has championed a theory of direct tissue vaporization such as is suggested for the rabbit. Loewy and Wechselmann (3) have reviewed with great detail the literature and they have presented studies on three men with congenital absence of sweat glands. It was shown that these subjects under conditions of rest and moderate surrounding temperature were able to eliminate through the skin approximately the same quantity of moisture that normal individuals eliminate and that under such conditions the rectal temperature did not rise. If however the temperature of the surrounding air was raised, or if the heat production of the subject was increased, then the water loss from the skin was less than in the cases of the normal individuals subjected to the same conditions and the subject's rectal temperatures rose. Associated with this rise there was always an increase in the minute volume ventilation of the lungs. The response of these subjects was quite similar to the rabbit under similiar conditions. The peripheral water loss from infants has been measured directly by McClure and Sauer (4). While no attempt was made by these authors to analyze the mechanism of this loss, they pointed out the customary absence of sweating in infants of the age of those investigated. It would seem therefore that man may probably make continuous use of this constantly acting method of water loss, and may call upon the discontinuous method (sweating) only when the heat accumulates in the body to such an extent as to initiate a rise in body temperature.

SUMMARY AND CONCLUSIONS

1. A resting fed rabbit can eliminate all of his heat as latent heat of vaporization if he be kept in an atmosphere of which the wet bulb temperature remains below 80°F.

2. A resting fed rabbit, although he is allowed to breathe relative cool (dry bulb 74°F.) and quite dry (wet bulb 56°F.) air, and although he does no rebreathing, cannot eliminate all of his heat through the respiratory surface.

3. A resting 2 kilo rabbit, which has recently eaten, and one from which food has been withheld for twelve to eighteen hours, when inclosed in a fireless cooker and ventilated in such a fashion that the rectal temperature does not rise, shows a heat production of 5.7 calories to 10 calories per hour.

4. It was shown to be theoretically improbable that a rabbit could develop sufficient ventilation to eliminate 5.7 calories per hour through the respiratory surface.

5. It must be possible therefore for the rabbit to eliminate significant quantities of heat through the peripheral surface in the form of latent heat of vaporization.

6. In the absence of sweat glands this water must be eliminated by a process of direct vaporization from the tissues. This water loss is to be sharply contrasted with water lost through the sweating mechanism. Sweat is a form of indirect water loss. It is separated from the tissues in the liquid phase and undergoes vaporization only after reaching the surface.

7. These data suggest that "insensible perspiration" in man is due to a similar direct vaporization of water from the tissues.

BIBLIOGRAPHY

(1) HUNT: Journ. Hyg., 1912, xii, 479.
(2) HALDANE: Journ. Hyg., 1905, v, 494.
(3) LOEWY AND WECHSELMANN: Virchow's Arch., 1911, ccvi, 81.
(4) MCCLURE AND SAUER: Arch. Int. Med., 1918, xxi, 428.

ON THE RELATIONSHIP BETWEEN THE GASTRIC ACID RESPONSE AND THE BASAL SECRETION OF THE STOMACH

ROBERT K. S. LIM[1]

From the Hull Physiological Laboratory, The University of Chicago

Received for publication April 18, 1924

In a recent investigation on the normal human gastric secretion, Lim, Matheson and Schlapp (11) found that the gastric response to a constant stimulus (histamine) varied directly with the basal secretion of acid. It is also known (7) that in Pavlov pouch dogs, the gastric response to a meal or other stimulus depends in some measure upon the state of the basal secretion, the amount of response obtained being greater when the basal rate is high and smaller when the basal rate is low. This dependence of the active state of the stomach on the basal has received no attention other than the utilization of the basal data as controls. For the purpose of qualitative observations, viz., the determination of whether certain substances do or do not excite the gastric glands, this suffices, but when comparative observations of a quantitative character are made, it is important that the exact relationship between the two functional conditions be known. For example, frequent repetitition of an observation on one animal with the object of obtaining a constant quantitative result is sometimes necessary. On each occasion that an experiment is made, however, the basal secretory rate will be found to be different, and similarly with the amount of response. Obviously, a conception of the true values in such cases can only be formed from a knowledge of the relationship existing between the basal and active phases of gastric secretion.

The present investigation is an extension of the one mentioned above on Pavlov pouch dogs. Histamine acid phosphate has again been employed (injected subcutaneously) in 1 mgm. doses, as the constant stimulus to evoke secretion. Only the acid of the gastric juice is analyzed and this in the usual manner using methyl orange and phenolphthalein as indicators. The figures given represent the *hourly* secretion and relate to the amount of juice, the percentage of total acid and the weight of acid.

THE BASAL SECRETION. The juice secreted by the stomach in the absence of all intentional and avoidable stimulation is termed the basal secretion.

[1] Rockefeller Foundation Fellow.

The cause of the basal secretion is as yet not definitely known, nevertheless it seems desirable that animals be kept under standard conditions, especially with regard to meals. In this work the dogs were not maintained under a strict regime, but in general bread and meat were given ad libitum once a day about 6 p.m. and the observations made in the morning. All records obtained after a normal or usual night's rest have been presumed to be basal, as it was aimed chiefly to work on dogs showing all ranges of basal secretion.

The criterion of the secretory state. It is the usual practice to employ the percentage acidity figure as the criterion of the gastric secretory state. The Russian school almost invariably uses the pepsin percentage. While this is unavoidable in many cases (viz., clinical test meal observations), in experiments on dogs and even in man (11), the total quantity of juice secreted is also obtainable from which the total output or weight of HCl (or pepsin) secreted can be computed. Since little or nothing is known regarding the relationship between the secretion of acid and of enzyme, and since determinations of the latter are less accurate, the secretory state of the stomach can for the present only be studied with advantage with reference to the former. Of the figures available, that representing the HCl output reflects most accurately the secretory condition insomuch as it is the product of the total (oxyntic) cell activity. Although it is not denied that the same information is afforded by regarding the quantity of juice and the percentage acidity together, the calculation of the HCl output permits of a more rapid and accurate estimate being formed. For example, the percentage acidity is known to be limited; it therefore follows that once this limit is reached the percentage fails to indicate further increases of the secretory stage. Again, the limitation of percentage allows of variations, especially in the direction of increase of the fluid constituent of the juice, without a corresponding increase in the HCl output. Thus, neither a large quantity of juice nor a high percentage necessarily implies a high secretory state, although normally such a construction is valid. These arguments are admittedly theoretical, but indicate the value of employing the HCl output figure as the criterion of functional activity and therefore as the basis of comparison.

It may be mentioned in passing that the HCl output is calculated from the total acidity, the free acid being observed mainly to note the occasions when it is absent.

The "normal" basal secretory rate. The rate of secretion of the basal juice may apparently vary widely, not only in different individuals but also in the same individual on different days. It would thus seem that all ranges of secretion rate may normally occur under basal conditions. From the recent observations made by Kunde (9) in fasting dogs, however, there is some doubt whether the higher basal rates can properly be con-

sidered under the above definition as strictly basal. It is therefore important to determine the secretory range which is most commonly exhibited.

In a series of one hundred and fifty-seven (daily) observations on twenty dogs, 89 per cent of the observations showed a secretion rate of less than 20 mgm. HCl per hour, while in 58 per cent of the cases the rate was below 5 mgm. HCl per hour. It is noteworthy that above 5 mgm. HCl, free acid is invariably present, while it is absent in 54 per cent of the cases below this rate. It is therefore significant that in every case some total acid was found in excess of the titratable value of distilled water to phenolphthalein. This is, however, not a fair comparison since the basal juice invariably contains relatively large amounts of mucus, and it is questionable whether such total acid as is found in these extremely small amounts can be taken to represent minimal quantities of HCl. In two dogs, suffering from acute distemper, almost no gastric secretion occurred under basal conditions nor did any increase occur following a meal, nevertheless, the juice available for estimation showed a slightly higher total acid value than an equivalent amount of water. These dogs could hardly be considered to be secreting acid. It thus seems not unlikely that the colloidal nature of mucus accounts (adsorption of alkali?) for the titratable total acid found with the lowest secretory rates. If this be the case, then some of the observed basal juices showing no free acid and containing less than 5 mgm. HCl (per hour) may reasonably be taken to represent a state of absolute achlorhydria or zero acid secretion. It would therefore follow that the secretion of HCl is not always continuous.

With reference to the higher basal rates the fact that only 11 per cent of the observations show a rate above 20 mgm. HCl per hour, indicates that these values must be accepted with reserve as being strictly basal. For the purpose of the present work, however, the question is immaterial.

The quantity and the percentage acidity of the basal juice at different secretion rates. In order to correlate the quantity of juice and the percentage of acid with the secretion rate, the values of the two former have been averaged at convenient intervals and plotted against the HCl output (see fig. 1). It will be noticed that with increasing HCl output, there is a proportionate increase of juice, the graph (termed the fluid graph) forming a straight line, except between the values 0 and 5 mgm. HCl. The percentage graph forms a curve corresponding to the ratio of fluid to acid. The fluid graph was empirically determined from the observed values, while the percentage graph was calculated on the basis of the former. The agreement between the observed and calculated values is satisfactory, considering the errors inherent in the method of experiment.

Two interesting points are brought out. One in relation to the deviation from the linear (fluid) graph occurring between 0 and 5 mgm. HCl, the

juice secreted being less than would be expected were the graph to continue as a straight line through this region. Attention is drawn to this in connection with the probability of the real zero of the secretory state falling somewhere between the observed 0 and 5 mgm. HCl; the deviation may well be false as the shifting of the zero to the right would mean that the fluid graph has a positive value at the true zero. Such an error would affect the whole graph but not to such an extent as would invalidate the conclusions drawn.

The second point of interest is that with higher secretory rates there is a rapid increase of percentage until 20 or 30 mgm. HCl per hour is reached

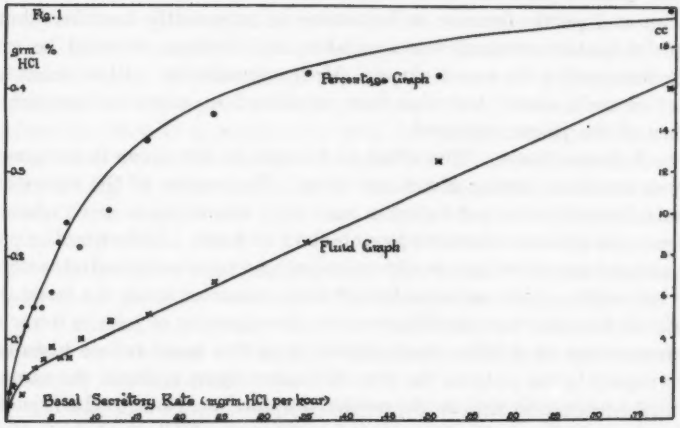


Fig. 1. Graphic representation of the relation between quantity of fluid, percentage acidity and secretory rate (output of HCl per hour) in the basal juice of the Pavlov pouch (Dog). The fluid graph is derived empirically from the observed figures while the percentage graph is calculated on the basis of the fluid graph and the HCl. output.

when the percentage curve flattens and approximates a straight line. This indicates that the proportion of fluid to acid, i.e., dilution and possibly neutralization, become minimal and relatively constant as secretion increases. The importance of considering the non-acid bearing fluid which embodies these factors (viz., surface mucous cell secretion, mucoid cell secretion, peptic cell secretion and possibly fluid which has diffused through the mucosa) is that it may be responsible for variations not only in the composition of the juice but also in the quantity. Whether HCl is secreted at constant or varying concentrations will be discussed later.

The course of the basal secretion. The basal secretion in the majority of cases continues uniformly when the secretion rate is low, i.e., below

20 mgm. HCl per hour. Above this rate it usually shows a gradual fall to a level below 20 mgm. It must not be taken, however, that hourly fluctuations do not take place, in fact the variations below 20 mgm. may commonly amount to ± 25 per cent, while above this rate the variations are relatively smaller, or they show, as already stated, a progressive decrease. In view of this it is important with the higher basal rates to observe the secretion for two hours in order to ascertain the tendency of the secretion state, while with the lower rates one hour observations are sufficient as the secretion is more or less stable.

THE ACTIVE SECRETION. Histamine has been used to stimulate the gastric glands, partly in order to confirm the observations already made in man and partly because in histamine an apparently harmless though powerful gastric excitant was available, and further, it could be given (subcutaneously) in exact doses. Meal stimulation (either meat and water or milk alone) has also been employed in order to compare the nature of the juices obtained.

The histamine juice. The effect of 1 mgm. of histamine is to cause an intense secretion lasting about one hour. The course of the secretion is essentially similar to that found in man (11), describing a curve when the analyses are made at shorter intervals than an hour. Following the phase of increased secretion there is approximately a return to the initial secretory rate but with a juice of entirely different character from the basal. In nearly all the observations (95 per cent) the quantity of juice is lower and the percentage of acidity much higher than the basal before histamine. With regard to the output, the post-histamine figure is about the same, as has just been mentioned, as the output obtained previous to the injection, when the initial secretion rate is below 20 mgm. HCl per hour. Below 5 mgm. the post-histamine output is sometimes higher, but above 20 mgm. it is always lower than the initial output. In short, there is after histamine a period of diminution (hereafter described as the post-histamine phase) which affects more particularly the fluid portion of the juice rather than the HCl. There is no apparent diminution of the HCl output except when the basal rate is high, although in this case it must be remembered that the secretory rate normally tends to fall.

If 1 mgm. of histamine is injected hourly, the secretion during the subsequent hours is distinctly lowered. It is diminished sometimes as much as 50 per cent of that of the first hour but does not cease even after many hours. Two and three milligrams have been injected hourly for eleven and seven hours respectively with similar results, i.e., without causing complete fatigue. If, however, the second injection is given two hours after the first, the usual response is obtained, i.e., one quantitatively equal to that evoked by the first injection. In passing, it may be stated that doubling or trebling the dose does not produce double or treble the response.

The diminution does not appear to be a direct effect on the cell, otherwise fatigue should have been observed. After having injected 2 mgm. hourly for ten hours, 1 mgm. of atropine sulphate was given with the histamine without producing more than a 33 per cent lowering of the secretion rate prevailing during the previous two hours. The dose of atropine given (the dogs being between 8 and 15 kilos) caused cardiac and pupillary symptoms and is well known to have an inhibitory effect on the motor function of the alimentary tract. Nevertheless, it has been repeatedly found, in confirmation of Keeton, Koch and Luckhardt (8), to have only a slight influence on a single injection of histamine. This slight lowering is explainable by the inhibition of the secretion caused by the same factors as maintain the basal secretion, since the normal range of basal secretion can invariably be completely inhibited by a similar dose of atropine. The practical inability to cause fatigue of the acid secreting (oxyntic) cells is supported by histological evidence, the oxyntic cells never exhibiting a complete depletion of granules such as is shown by the peptic cells after prolonged activity. The present observations confirm those of Rothlin and Gundlach (15), who also failed to cause fatigue of the acid secreting apparatus with histamine. They are also in line with the findings of Bergeim, Rehfuss and Hawk (1), who employed water stimulation, and of Ivy (5) who used both water and gastrin. The cause of the post-histamine diminution can probably be referred to the vascular changes described by Dale and others (3). At any rate the possibility of vascular changes influencing the volume of the juice secreted apart from the secretory state, merits consideration.

The quantity and the percentage acidity of the histamine juice at different secretion rates. In order to construct a graph which would cover all ranges of HCl output, the analyses of both the histamine juice (from single and repeated injections) and the post-histamine juice have been utilized (see fig. 2). The quantity of juice and the percentage acidity have been plotted against the corresponding HCl output, as was done with the basal juice.

The fluid graph is a straight line differing from the basal in two respects. First, in the region between 0 and 12.5 mgm. HCl. The graph instead of forming a curve as it is traced to zero, proceeds as another straight line after departing from the main portion of the graph at 12.5 mgm. In consequence, the percentage acidity remains practically constant throughout this region. The observed percentage values fluctuate above and below the calculated as might be expected if the percentage was constant. The observations recorded here are all of the post-histamine phase and are not necessarily related to the remainder. They serve, however, the purpose of comparison with the similar region of the basal.

As the secretion rate, during the post-histamine period, has returned approximately to the initial basal rate, the smaller amount of juice must be due to diminution of the non-acid bearing fluid. The high acidity of the post-histamine juice is important as it serves as a clue to the concentration at which HCl is secreted. If the acid concentration leaving the cell increases with the secretory rate, it would necessarily follow that with an infinitesimal rate of secretion, the concentration must be not less (on the average) than 0.294 per cent, since this is the percentage found in the post-histamine juice, while it would not be much more than 0.6 per cent at the highest possible rate, as this is the known physiological limit.

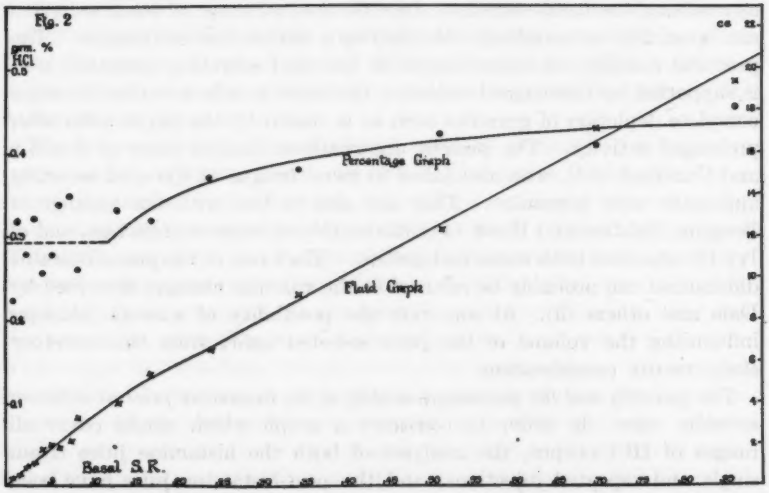


Fig. 2. Graphic representation of the relation between quantity of fluid, percentage acidity and the basal secretory rate (S. R.) on the histamine juice (Pavlov pouch dog).

(The figure 0.6 per cent is only obtainable when the gastric juice is collected at the height of an intense stimulation. In the present work it must be remembered that the figures given are for hourly collections.) If the acid is secreted at a constant concentration, the percentage is required to be higher than 0.6 per cent in any state of activity. It will be recalled that the percentage curve of both the basal and the histamine juice (that of the meal juice might also be added) commences to flatten out approximately after the secretion rate has passed 30 mgm. HCl per hour. The "increased concentration" hypothesis would therefore operate chiefly in the region 0 to 30 mgm. HCl and an increase of secretion rate from mini-

mum to maximum would merely mean an advance of the true concentration from 0.294 to 0.6 per cent under the most favorable conditions. Both hypotheses demand that the acid leaves the cell at high concentration, while the increases supposed to occur according to the first hypothesis are sufficiently accounted for by the variations in the non-acid bearing fluid. Pavlov (14) has suggested that HCl is neutralized to a greater extent by mucus when the secretion rate is low, on the assumption that more time is given for this to take place. This is obviously untenable, since pyloric and probably fundic mucus is almost neutral (6), (10). In fact a sufficient amount of alkali to permit of the observed differences of acidity has not been demonstrated. Foster and Lambert (4) found that the total chlorides of dog gastric juice vary with the secretion rate. Carlson (2) and Lim, Matheson and Schlapp (12) have observed a similar relation in man. This has been interpreted to mean that the concentration of HCl secreted varies with the secretion rate. In the above cases, however, the quantity of juice is taken as the criterion of the secretory rate. While this is more or less valid, its usage neglects the consideration of the non-acid bearing fluid. At low secretion rates (HCl output) the concentration of mucus is unquestionably higher, especially in the case of the entire stomach juice, containing the pyloric secretion, which is continuous and little altered by secretory activity in the fundus. Furthermore, Carlson found that the total solids are more concentrated in the "hunger" than in the "appetite" juice, the former being the basal juice secreted at a low rate. Pepsin is always present even at the lowest (acid) secretory rate observed. In short, the evidence in favor of dilution has hitherto been overlooked because of the adoption of the juice quantity as the criterion of secretory rate.

The second point of difference between the histamine and basal fluid graphs is in the greater rise exhibited by the former at the higher secretory rate, indicating that the histamine juice is greater in volume but lower in percentage. This is again interpreted in terms of non-acid bearing fluid, which is seemingly increased at the height of stimulation.

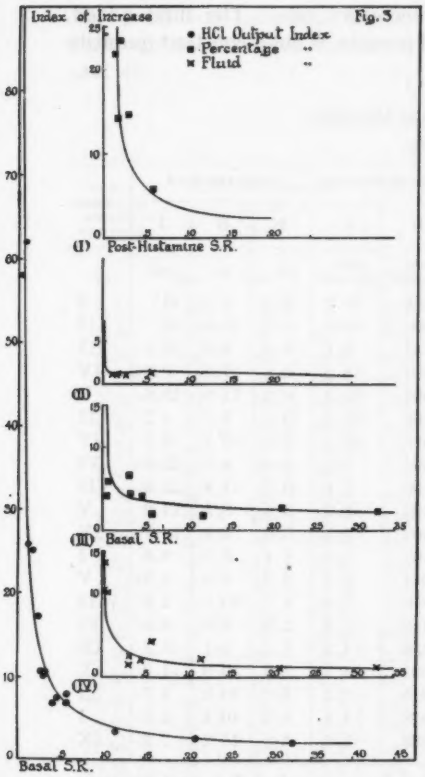
The effect of histamine thus has two phases, one in which increased volume of juice is observed followed by the reverse. As far as can be ascertained, the volume changes are independent of the secretory rate. If, as has been suggested, the volume changes are dependent on blood flow, then histamine must cause at first increased circulation through the stomach and later a diminished blood flow. That stasis from capillary dilatation and arteriole constriction is the characteristic effect of histamine on vessels is known (3), and would result in a reduced blood flow. At an early stage of the capillary dilatation, however, before a marked fall of blood pressure occurred, the increased calibre of the peripheral vessels would lead to increased blood flow. According to Mainwaring, Monaco

and Marino (13), histamine causes vasoconstriction and edema in the isolated dog gut. This shows that some change of this kind may be expected to occur in the gastric vessels, although not to the extent of edema in the intact living animal, and with the dose given. In man the subcutaneous injection of 1.5 mgm. of histamine causes sufficient vasodilatations to be recognised subjectively; in the dog it is assumed that 1 mgm. (subcutaneously) will have the same effect.

The relation between the active response and the basal secretory rate. In order to compare the amount of response obtained at various basal rates, Lim, Matheson and Schapp employed the ratio $\frac{\text{response}}{\text{basal}}$ which was termed the *increase index*. They found that in the case of man, the HCl output and the percentage increase indices varied inversely with the basal secretory rate, while the juice index varied inversely up to a point, and that between zero secretion and this point, it varied directly with the basal rate. This has also been found to be generally true for Pavlov pouch dogs (compare figs. 3, 4 and 6). It will be convenient, however, to deal with the increase of output, percentage and juice separately.

The increase of HCl output after histamine. As the histamine output index varies inversely with the basal output, it indicates that the output response might be in some way constant, i.e., it either attains a constant figure, regardless of the basal rate, or it varies in a parallel fashion with the basal. The latter has been found to be more nearly the case. By deducting the basal HCl output from the histamine output a tolerably constant figure is obtained. In one series of twenty observations on eleven dogs, the mean of this value was found to be 35 mgm. HCl, the standard deviation being ± 6 mgm., and in another series of twenty-one observations on six other dogs, 72 mgm. HCl, the standard deviation being ± 15 mgm. (tables 1 and 2). The difference in the two series is attributed to the size of the pouch rather than the size of the animal, because extremes of weights occurred in both series and the values are progressive. The division of the observations into two series was quite arbitrary, and was determined mainly by the fact that the frequency was distributed in a like manner about the mean of the total observations. That the size of the pouch is the most important factor is evident when it is considered that the stimulus affects the whole stomach. Unfortunately, there are no means at present of making a uniform size pouch; the proportion of pouch to whole stomach in the above animals cannot be exactly estimated, but judging from the extent of the procedures at the time of operation, pouches of about $\frac{1}{6}$ to $\frac{1}{3}$ of the entire organ were constructed. Other factors are the state of "tone" of the glands, small errors in the amount of drug injected, the rate of absorption of the drug, the concentration of the drug in the blood stream, and the state of health of the animal. Excepting

the last, these factors are practically uncontrollable; their influence is on the whole not considerable, although quite evident from the variations shown by the same animal. Accepting the mean values as the constant for each series, the histamine output index may be calculated as follows:



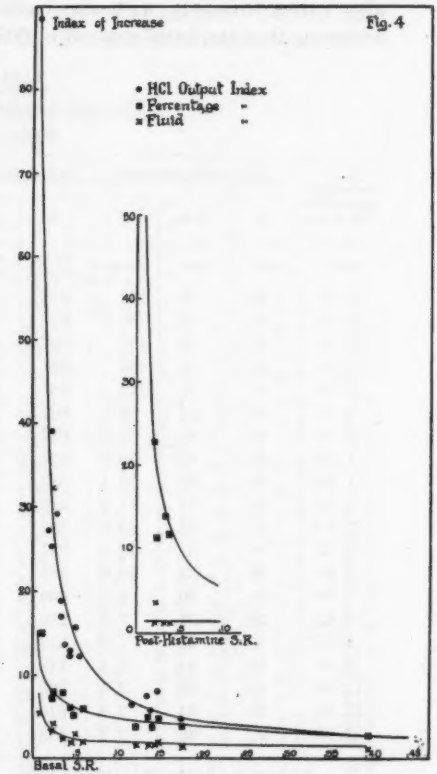


Fig. 3. Curves showing the increase of HCl output, HCl percentage and fluid obtained at different basal secretory rates, after an injection of histamine. The two upper curves (I and II) show the increase of percentage and of fluid at varying post-histamine secretory rates. Table 1, series A, (Pavlov pouch dog).

Fig. 4. Similar curves to those shown in figure 3, taken from table 2, series B, (Pavlov pouch dog).

Since it is the sum of the initial basal output and the output called forth by one milligram of histamine, and the index is represented by the ratio $\frac{\text{response}}{\text{basal}}$, by substituting the real values, $O = \frac{K + x}{x}$ is obtained, where O

is the index, K the constant and x the basal HCl output. The graph of this function is thus a rectangular hyperbola. The agreement between observed and calculated values may be compared in figures 3, 4 and 6.

The increase of juice and percentage acidity after histamine. The increase in the amount of juice after histamine, as indicated by its increased index, also varies inversely with the basal secretory rate. The fluid added, assuming that the basal amount of fluid persists, is not a constant quantity

TABLE 1

The acid response to histamine

Series A

BASAL SECRETORY RATE HCl PER HOUR	HCl OUTPUT PER HOUR			HCl PERCENTAGE PER HOUR			FLUID PER HOUR			DOG
	H	H-B	I	B	H	I	B	H	I	
mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc.*	*cc.*	*cc.*	
0.04	29	29	725	018	355	19.7	0.2	8.2	41	I
0.05	38	38	760	027	292	10.8	0.2	13.0	65	II
0.22	28	28	127	037	337	9.1	0.6	8.3	13.8	III
0.22	35	35	159	027	492	18.2	0.8	7.1	8.9	IV
0.30	43	43	143	037	392	10.3	0.8	11.0	13.8	V
0.62	35	34	58.3	027	365	13.5	2.3	9.7	4.2	II
0.74	46	45	62.2	037	375	10.1	2.0	12.2	6.1	IV
1.31*	34	33	26.0	328	392	1.2	0.4	8.8	22.0	VI
1.91*	48	46	25.1	382	406	1.1	0.5	11.8	23.6	VII
1.92*	33	31	17.2	320	392	1.2	0.6	8.5	14.2	V
2.95*	32	29	10.8	492	369	0.8	0.6	8.8	14.7	IV
3.32	34	31	10.2	237	362	1.5	1.4	9.3	6.6	I
3.41	36	33	10.6	155	374	2.4	2.2	9.5	4.3	V
4.36	34	30	6.9	109	319	2.9	4.0	10.5	2.6	VIII
4.82	36	31	7.5	219	410	1.9	2.2	8.8	4.0	VI
5.97*	42	36	7.0	373	456	1.2	1.6	9.1	5.7	IX
6.14	50	44	8.1	118	501	4.2	5.2	10.0	1.9	X
12.00	42	30	3.5	200	415	2.1	6.0	10.0	1.7	XI
21.42	55	34	2.6	510	528	1.1	4.2	10.4	2.5	I
32.84	73	40	2.2	483	483	1.0	6.8	15.2	2.2	IX

H = histamine juice; B = basal juice; I = increase index; * = post-histamine juice.

but shows a slight increase with high basal secretory rates. This again shows that the histamine juice contains more fluid than the basal at equi-secretory states.

The percentage acidity being a function of both the HCl output and the quantity of juice, its increase is necessarily determined by the relation of these two factors. The percentage increase index may thus be represented as $P = \frac{O}{F}$ (where P = percentage index, O = output index, and

F = fluid index), from which it follows that $F = \frac{O}{P}$ and $O = FP$. This shows clearly the relation of the three indices to one another; it also permits of the output index being obtained without direct calculation of the output, on the basis of the theoretical output index and of the graphs of the basal and histamine juices. The curves of all three indices have been constructed and compared with the observed values (see figs. 3 and 4).

TABLE 2

The acid response to histamine

Series B

BASAL SECRETORY RATE HCl PER HOUR	HCl OUTPUT PER HOUR			HCl PERCENTAGE PER HOUR			FLUID PER HOUR			DOG
	H	H-B	I	B	H	I	B	H	I	
mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc.*	*cc.*	*cc.*	
0.32	88	88	275	027	552	20.4	1.2	16.0	13.3	XII
0.44	80	80	182	055	392	7.1	0.8	20.5	25.6	XIII
0.95	84	83	88.4	073	410	5.6	1.3	20.5	15.8	XIV
2.02*	56	54	27.2	337	410	1.2	0.6	13.7	22.8	XIII
2.36*	92	90	39.0	118	411	3.5	2.0	22.5	11.3	XV
2.49	63	61	25.3	119	401	3.4	2.1	15.6	7.4	XVI
2.55	82	79	32.2	091	383	4.2	2.8	21.5	7.7	XV
3.01*	88	85	29.2	301	438	1.5	1.0	20.0	20.0	XIII
3.55*	60	56	16.9	355	428	1.2	1.0	14.0	14.0	XV
3.63	68	64	18.8	173	410	2.4	2.1	16.5	7.9	XIV
3.91*	53	49	13.6	355	410	1.2	1.1	13.0	11.8	XIII
4.62	59	54	12.8	210	419	1.9	2.2	14.0	6.4	XIV
4.64*	57	52	12.3	309	455	1.5	1.5	12.5	8.3	XV
5.08	79	74	15.6	127	382	3.0	4.0	20.6	5.2	XVI
6.13	75	69	12.2	219	456	2.1	2.8	16.5	5.9	XIV
12.30	80	68	6.5	292	419	1.4	5.0	19.0	3.8	XIV
13.85	104	90	7.5	301	465	1.5	4.6	22.3	4.8	XIII
14.60	84	69	5.8	292	456	1.6	5.0	18.5	3.7	XIV
15.09	125	110	8.2	256	447	1.7	5.9	28.0	3.7	XIV
17.99	87	69	4.8	346	447	1.3	5.2	19.5	3.8	XVII
40.29	107	67	12.7	474	456	1.0	8.5	23.5	2.8	XV

H = histamine juice; B = basal juice; I = increase index; * = post-histamine juice.

The acid response during the post-histamine phase. If histamine is given two hours after a previous injection, that is, during the post-histamine phase, a similar quantitative but not qualitative change is obtained. At secretion rates between 0 and 12.5 mgm. HCl, the fluid increases are considerably higher (than at corresponding basal rates) and the percentage indices lower. With the additional difference that the percentage index curve is not only more rectangular but also shows a characteristic "delay."

The difference is due solely to the nature of the post-histamine juice (see figs. 3 and 4), and is the counterpart of that observed in man (fig. 6).

The meal secretion. The juice secreted to a meal has been correlated with the secretion rate in order to compare it with the juice secreted under basal conditions and as a result of the histamine stimulus. It is found that the meal juice occupies an intermediary position between the former and latter, being identical with the basal juice in all respects except the volume of the fluid at the higher secretion rates (see fig. 5).

A comparison with the human gastric secretory function. With regard to the acid response, the results obtained in man (11) have on further analysis been found to be similar to those obtained in the dog. That is to say, the observed output indices agree with those calculated from the

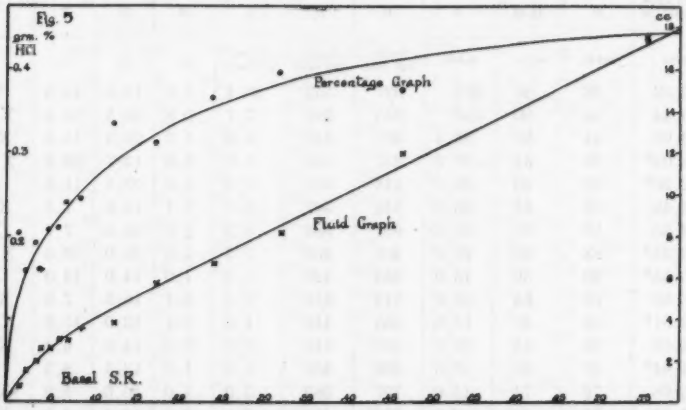


Fig. 5. Graphic representation of the relation between quantity of fluid, percentage acidity and basal secretory rate in the meal juice (Pavlov pouch dog).

formula $O = \frac{K + x}{x}$, the value of the constant being 73 mgm. HCl; it must be admitted, however, that the standard deviation is larger (± 20 mgm. HCl).

The percentage increases bear the same relations to the basal secretion as in the dog, with the exception that in man the percentage index curve is less rectangular. As a result of this, the juice index curve exhibits a "delay" (see fig. 6) within the region of low secretion. Since the juice increase index depends on the ratio $\frac{O}{P}$ the deviation is evidently due to the presence of a higher value of P than obtains in the dog at the corresponding region. This also means that the percentage acidity of the

human basal juice is lower (relatively) and the quantity of juice higher, than in the dog at the minimum secretion rates. In point of fact the nature of the empirical curves require that the quantity of juice be almost constant, instead of diminishing toward zero secretion. From the analysis of the human basal juice, it was found that the fluid curve would not proceed to zero. The actual juice values show much larger variations than are observed in the dog, so that it is not possible with the number of observations available, to obtain a graph with any degree of accuracy. Nevertheless, it is certain that the true graph would exhibit a certain quantity of fluid at zero HCl output and with increase of secretory rate, at first relatively small increases of juice (up to about 15 mgm. HCl per hour) and later progressively larger. These conditions satisfy the demands of the index curves.

The difference, then, between man and dog lies not in the response but in the composition of the basal juice at the lowest secretory rates (cf.

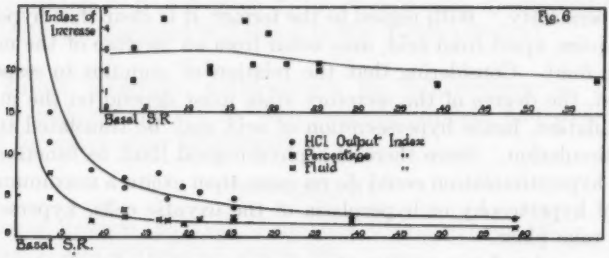


Fig. 6. Similar curves to those in figures 3 and 4 taken from human experiments

response during post-histamine phase). In the human observations the entire stomach juice was collected at intervals of ten minutes. The inclusion of the pyloric secretion with that of the fundus introduces a large element of non-acid bearing fluid into the composition of the juice analyzed. Consequently, at the lowest secretion rates the basal juice is composed almost entirely of mucus (chiefly pyloric) and such additions of HCl as would occur from zero upwards to a certain point would make a very little difference to the quantity measured. With higher secretion rates, on the other hand, the pyloric factor would become less evident. The explanation of the more or less constant quantity of the human basal juice at low secretion rates may, in part, however, be found in the suggestion that mucus introduces an error in the titration value. This error would be much larger in the human estimations on account of the preponderating amount of mucus which is present at the secretion rates under consideration, so that the probability of the true zero of secretion lying between

the observed O and say 12.5 mgm. HCl is equally great. That is to say, the spreading out of juice values over a larger range of secretion than is actually the case may make for a constancy which is more apparent than real. At any rate, the difference between the secretory response of man and that of the dog cannot be regarded as fundamental.

CONCLUSIONS. The results obtained show that quantitative observations on gastric secretion are possible, provided the basal or the initial conditions previous to the actual experiment are observed. A word of warning is necessary regarding the comparisons of meal or other orally administered stimuli. Here, the influence of varying motor conditions of the bowel will almost certainly alter the quantitative presentation of the stimulus and thus affect the amount of gastric response.

The importance of employing the HCl output as the criterion of the secretory state is considered to have been sufficiently demonstrated to justify its more universal use.

The present results also have a bearing on the questions of hypersecretion and hyperacidity. With regard to the former, it is clear that hypersecretion of juice, apart from acid, may occur from an increase of the non-acid bearing fluid. Considering that the relation of stimulus to response is constant, the degree of the secretory state must depend on the intensity of stimulation, hence hypersecretion of acid may be translated to mean hyperstimulation. Since there is a physiological limit to function, however, a hyperstimulation could do no more than excite a maximum effect. Short of hypertrophy or hyperplasia of the oxyntic cells, hypersecretion cannot take place.

With regard to hyperacidity, there is first of all the fact that no higher figure than 0.6 per cent has ever been observed to occur. This limitation of the percentage is probably connected with the maximum capacity of the cells to secrete, for there is no reason other than the physiological limit to function, to prevent the concentration from being higher. The concentration of HCl increases by such small increments after a comparatively low secretion rate is attained that in order to have a higher percentage than this, i.e., hyperacidity, the secretion rate must be increased much beyond the maximum capacity to function. In short, if hyperacidity is physiologically impossible, so is hypersecretion of acid, and vice versa.

It may be stated, however, that a meal does not usually tax the stomach to any extent, a maximum secretion being extremely rare. This, of course, permits of variations up to the maximum, which compared with the usual, simulates a hypersecretion. In such cases the factor of difference lies, not in the stomach, but at the site of stimulation.

SUMMARY

1. The only reliable criterion of the rate of acid secretion in the stomach is the weight or output of HCl secreted per unit time.

2. The composition of the gastric juice, whether basal or active, varies with the secretory rate, the quantity of juice increasing uniformly with the output of HCl. The increase of percentage is rapid at low secretion rates and relatively constant at higher rates. The active juice differs from the basal in the quantity of fluid present at equal secretory rates.

3. The acid response to any stimulus varies directly with the basal secretory rate, being the sum of the acid secreted at the initial basal rate and the acid evoked by the stimulus, the latter being constant for the stimulus.

4. The fluid response is not constant and is the factor which may vary the character of the response to different stimuli.

5. Such questions as the influence of vasomotor changes on secretion, the concentration of HCl secreted by the oxyntic cells, hypersecretion and hyperacidity are discussed in relation to the results obtained.

The author gratefully acknowledges his indebtedness to Doctors Carlson, Luckhardt and Ivy for their valuable criticism and coöperation and also for many other kindnesses.

BIBLIOGRAPHY

(1) Bergeim, Rehfuss and Hawk: Journ. Biol. Chem., 1914, xix, 345.
(2) Carlson: This Journal, 1915, xxxviii, 248.
(3) Dale and Laidlaw: Journ. Physiol., 1910, xli, 318; Dale and Laidlaw: Ibid., 1911, xliii, 182; Dale and Richards: Ibid., 1918, lii, 110.
(4) Foster and Lambert: Journ. Exper. Med., 1908, x, 820.
(5) Ivy: This Journal, 1918, xlvi, 420.
(6) Ivy and Oyama: This Journal, 1921, lvii, 51.
(7) Ivy and McIlvain: This Journal, 1923, lxvii, 124.
(8) Keeton, Koch and Luckhardt: This Journal, 1920, li, 469.
(9) Kunde: This Journal, 1924, lxviii, 389.
(10) Lim and Dott: Quart. Journ. Exper. Physiol., 1923, xiii.
(11) Lim, Matheson and Schlapp: Ibid., 1923, xiii, 333; Ibid., 361.
(12) Lim, Matheson and Schlapp: (unpublished).
(13) Mainwaring, Monaco and Marino: Proc. Soc. Exper. Biol. and Med., 1922, xx, 182.
(14) Pavlov: The work of the digestive glands. London, 1910, 2nd ed.
(15) Rothlin et Gundlach: Arch. intern. de physiol., 1921, xvii, 59.

STUDIES IN BLOOD DIASTASE

II. Are the Blood Diastases End Products of Metabolism?

SEYMOUR J. COHEN

From the Department of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago

Received for publication April 18, 1924

In a previous article (1) it was shown that these enzymes have a relation to certain types of hyperglycemia, and possibly have some specific function in the carbohydrate metabolism. In this paper I wish to record experiments which indicate that the diastases are not end products of metabolism, as several investigators have suggested (2), (3), (4).

There are several reasons which render this view somewhat unacceptable. If diastase be an end product of metabolism, we should expect it to increase in the blood serum of animals which have undergone marked muscular exertion, or in which there has been an increase in the body metabolism. Such, however, is not the case.

We have performed three different types of experiments in which we have increased the metabolism of the animal and studied the blood diastase, and in no case have we obtained results which indicate that diastase is a breakdown product.

These experiments include: *1*, marked muscular exertion, due to drugs (strychnine); *2*, fatigue caused by work; *3*, fatigue due to non-specific fever.

Experiment I. A normal dog which was starved for 18 to 24 hours was used. A normal blood sugar and diastatic activity of the blood was determined. Then a dose of strychnine, just large enough to produce muscular twitching and slight convulsions, but not large enough to produce death, was given subcutaneously. The blood sugar and diastase were determined at 2-hour and 4-hour intervals, after the onset of the tremors, which lasted about 3 hours.

RESULT	BLOOD SUGAR	BLOOD DIASTASE
Before	0.150	62.4
Injection of strychnine:		
2 hours	0.200	57.4
4 hours	0.214	49.1

These results show that strychnine produced a moderate hyperglycemia with a decrease in the blood diastases.

Experiment II. This consisted of fatiguing a normal dog by letting him run on a treadmill and carrying a 1 to 2 pound weight. The speed of the treadmill was about 2 miles per hour—a rather fast walking gait for a dog. The blood sugar and diastase were determined before and after 4 hours of running.

RESULT	BLOOD SUGAR	BLOOD DIASTASE
Before	0.093	53.8
4 hours later	0.103	35.8

These results show only a slight increase in blood sugar, with a marked drop in the blood diastase.

Experiment III. In this experiment we subjected a dog to a hot box and elevated his temperature several degrees, then studied the changes in the blood diastases.

RESULT	TEMPERATURE	BLOOD SUGAR	BLOOD DIASTASE
Before	103.2	0.136	66.4
1 hour later	107.0	0.166	63.0
3 hours later	102.8	0.150	65.0

These results show only a slight increase in the blood sugar with practically no change in the blood diastase.

These three types of experiments are different methods of increasing the body metabolism: first, by certain drugs; second, by fatigue; third, by non-specific fever. Now, if this diastatic enzyme be an end product of metabolism and is poured into the blood stream to be eliminated by the kidneys, then we should expect to find an increase in the blood; but in contrast, we find a decrease in the blood. According to the theory which we stated in a previous article, we should expect, and do find, a decrease in the blood diastase, because any condition which causes an endogenous hyperglycemia will also cause a fall in the blood diastase, which is due to a mobilization of the circulating diastase to the liver and other organs, where the glycogen is being transformed to glucose.

SUMMARY

From our previous observations, and especially from these three types of experiments, we must conclude that the diastases in the blood are not entirely useless nor end products of metabolism; but they are circulating

in the blood, some of which must be eliminated by the kidney, but the greater portion of which lie in readiness to be used in cases of emergency or increased need for their action.

BIBLIOGRAPHY

(1) COHEN: This Journal, 1924, lxix, 125.
(2) CARLSON AND LUCKHART: This Journal, 1908, xxiii, 148.
(3) KING: This Journal, 1914, xxxv, 301.
(4) OTTEN AND GALLOWAY: This Journal, 1910, xxvi, 347.

COLOR PHENOMENA CAUSED BY INTERMITTENT STIMULATION WITH WHITE LIGHT

G. N. STEWART

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University

Received for publication April 19, 1924

In 1885, working in the Physics Laboratory of Edinburgh University, I observed and studied the succession of colors produced when the retina is intermittently stimulated by white light, and the rate of intermission, always inferior to that required for complete fusion, is progressively increased. It was shown that for any given intensity of the light (except when it is excessively strong) there is a rate of intermission with which the violet preponderates; and that with a higher rate the green preponderates—with a still higher speed the red. With suitable illumination and a rate gradually increasing from that which suffices to bring out the violet, the color becomes bluish violet, then bluish, then bluish green and finally green. Under appropriate conditions the predominance passes from green to yellowish green and yellow with further increase in the rate. So that it may be said in general that with white light of given intensity the preponderating subjective color sensation passes progressively from the violet toward the red, approximately in the order of the spectral colors, as the rate of intermission is progressively increased. This was also demonstrated by intermittent observation of the spectra of the lights employed.

It was further shown that with a given constant rate of intermission the same succession of colors can be obtained by varying the intensity of the light, an increase of objective intensity causing the preponderating subjective color to shift toward the violet end and a decrease of objective intensity causing it to shift toward the red end.

The observations were made by looking at the band of light representing the successive images of a source of light (gas-light, sunlight, candle-light or the light of a petroleum lamp) reflected from a mirror rotating at a known rate. A mirror silvered on the front was used as well as one silvered on the back, and there was no essential difference in the appearances. The luminous band was usually a broad one (12 or 15 mm. or more), so as to separate possible changes due to simultaneous contrast at the edges from the color changes in the band as a whole. As a matter of fact, it

was shown that even at the edges the color phenomena were essentially the same as in the rest of the band, but differed in phase because of the smaller intensity of illumination of the edges. Many experiments were made without the mirror, the source of light, or objects illuminated by reflected light, being viewed through an opening on the circumference of a blackened disc, which was rotated at a variable rate. Most interesting color effects can be obtained in this way by observing a well-lighted picture (reproduced in black and white) through the revolving disc.

It was recognized that contrast, or more generally changes evoked by excitation in the neighborhood of a directly excited area of the retina, might take some share in the production of the color effects under discussion, especially, of course, when the field contained a pattern favorable to the development of contrast phenomena, for instance, a picture with numerous fine lines or dots not too close together. But even here it is not difficult to observe that in general the color phase shown by one or other portion of a picture reproduced in black and white, with a given rate of intermission, is determined above all by the objective intensity of illumination of the given portion, that is to say, the depth of the shading, so that shaded parts stand out in colors nearer the red end of the spectral scale than white parts or than less shaded parts. It is easy to cause the darker foliage in an engraving of a landscape to assume a green color while the less shaded water or sky is violet or bluish, with the details of the shading represented by corresponding nuances of the subjective color. Then by a change of speed of the disc a complete change can be produced in the colors, which again, however, are appropriately placed on the different parts of the picture according to the depth of the shading. The colors are so easy to obtain that I have seen children amuse themselves by the hour "coloring" pictures in books or newspapers subjectively by observing them through a rotating disc, and describing the colors correctly. While it is generally not difficult to clothe landscapes in colors which are appropriate, or at least, not glaringly incongruous, it is of course different with pictures in which human figures or faces are the important element. With the shading below the chin perhaps a light green (at a particular speed of rotation), the cheeks violet or bluish, the hollows of the eyes perhaps green, the hair a dark green, and the various garments colored according to their shading (creases, for instance, at a given phase green), and then everything changing phase with increase or diminution in the speed, the personal appearance of a stage beauty, the statesman or criminal of the hour, the horse which has just won the Derby, or the reigning pugilist, is apt to be somewhat bizarre, and therefore rather amusing for children. On the other hand black and white pictures of such objects as embroidery, tapestry, Scotch tartans, carved work, stained and cut glass, etc., may show curious and even beautiful appearances, the subjective

colors imparting a certain richness to the designs. Of course, unless the black and white pictures were expressly produced in order that the subjective tints might approximate to the actual colors at some rate of intermission (a feat perhaps in simple cases theoretically possible), the subjective colors could only by accident be correct.

The colors beyond the green toward the red end are in general, under our experimental conditions, less strikingly elicited and more impure than those toward the violet end, because as the rate of intermission is increased the fusion point, at which the object appears as if illuminated steadily by the white light, is always coming nearer. A good deal depends upon the intensity of illumination of the picture or other object and upon the breadth of the open sector. But while the successive predominance of violet, blue and green is always easily, and, under proper conditions, brilliantly demonstrable, and while a more or less satisfactory yellow can be seen to succeed the green phase, there are usually beyond this only reddish brown tints (very far from a saturated red) before the black and white of steady fusion is attained. With suitable experimental conditions the predominance of the red tints can be clearly elicited.

Numerous observations were also made with colored lights both with the rotating mirror and by observation through the blackened disc. It was shown that the changes obtained subjectively in the colored images (and these can be strikingly elicited by viewing well-lighted colored pictures through an opening in the rotating disc) agreed, on the basis of the Young-Helmholtz theory, with those observed with white light. With colored pictures the blue tints are enhanced and white parts become violet with slow intermission; while the greens and especially the reds are weakened. With an increase in the rate blue remains conspicuous, and the greens begin to prevail. With a still higher speed the red parts emerge more and more. Finally, of course, with complete fusion the picture is seen in the same tints as if looked at without intermission. All gradations are seen in the color changes in the different parts of the picture according to the actual objective hues, their brightness, and their degree of saturation. The Purkinje phenomenon is not a factor in the changes. Intermittence of the light is the essential thing: given this, the characteristic changes are obtained with very different intensities of illumination.

After an interval of two years the experiments were continued in the Physiological Laboratory of Owens College, Manchester, and the results were published in 1888 in the Proceedings of the Royal Society of Edinburgh (1). The explanation suggested for the phenomena was that "when the retina is stimulated by a succession of very short flashes of white light, the proportion between the amount of excitation in the three hypothetical groups of fibres is not constant. With a certain duration of each stimulus, the excitation in the violet group preponderates; with a

shorter duration, that in the green preponderates; with a still shorter duration, that in the red. This statement so far constitutes merely a convenient way of summarizing observed facts." As to how the preponderance of one or the other color sensation is actually brought about, it may be assumed either that the curve which represents the time relations of the rise of the excitation to its maximum differs for each primary color in such a way as to permit the observed preponderance, or that the difference is in the time relations of the decline of the excitation, or that the difference extends both to the waxing and the waning of the sensation. In the paper it was assumed that the curves differ both in the ascent and in the descent, the color changes observed by Helmholtz, Fechner and others in the positive after image of a white object while it is disappearing being adduced in support of the view that there is a difference in the curves representing the decay of the excitement. McDougall's result, that the "action time," (the minimum time for which a luminous stimulus must last in order that the intensity of the sensation shall be equal to that produced by the light when it acts steadily) is the same for different colored lights giving equal luminosity sensations, does not seem definitely to exclude differences in the rise of the fundamental sensations as causes of the color effects under discussion. For in the first place I do not understand that there is proof that the form of the curves may not vary, and secondly the luminosity of the spectral components of the white light is of course not equal. But for our present purpose the matter is of no consequence. If the fundamental sensations develop in precisely the same proportional intensity with each rate of intermittance as in the steady stimulation, and the resultant sensation is that of white light during the period of ascent, the whole difference must develop during the period of decay, including, of course, the "pulsatory" phases of the after images, described by Hess (2) and afterwards by Bidwell (3), at least to the extent that there is time for these to intervene in the short period of relative darkness succeeding each stimulus.

Nearly 10 years after my experiments Bidwell, (4), Liveing (5), Abney (6) and others made similar observations on the colors produced by the so-called Benham's "artificial spectrum top" (7), introduced as a toy about 1894. Pieron (8) has recently returned to the question of the origin of the colors seen with Benham's top. Curiously enough, not one of these writers makes any reference to my work, although the phenomena studied by them are obviously of the same nature, but not, I think as well shown on the top as by one or other of the arrangements employed by me, and certainly no more simply. Further, at least three of these writers, including the most recent of them, emphasizes essentially the same factors as I did in attempting an explanation, although undue weight is sometimes given to a single factor to the exclusion of others which may, and probably do act concomitantly. Another of the writers referred to (4), who denies that these factors are important, was misled, I believe, by the character of the objects used in his study, including the "artificial spectrum" top, into attributing too large a rôle to a factor which, however it may

influence the subjective colors seen with particular patterns, and with illumination of small retinal areas, can be shown, by choosing the conditions properly, to be unessential. Even for the special case of Benham's top, I do not think that any of these writers has quite satisfactorily grappled with the details of the color changes.

Thus, Abney thought that the subjective color phenomena shown by the top are sufficiently accounted for on the hypothesis that the order of persistence of the three fundamental sensations is violet (blue), green and red. Liveing explained them on the assumptions that different spectral lights composing white light require different times to produce the corresponding sensation, the necessary period being shortest for red, and that the persistence of the impressions also differs, blue being the last to disappear. Pieron, who speaks of Benham as having rediscovered the phenomenon in 1894, states that his own systematic study (in 1922) has enabled him to penetrate the mechanism of production of the colors, and that the fundamental law of the phenomenon can be formulated thus: "Under the influence of a luminous excitation of the retina by a complex radiation giving a colorless resultant, there is produced at the beginning a 'deséquilibre chromatique' with successive predominance of nuances proceeding from red to blue in the order of the spectral colors, in consequence of an unequal velocity of establishment, up to the attainment of the transitory hypermaximal stage, of the fundamental chromatic processes." By the transitory hypermaximal stage he means a preliminary rise in the luminosity of the sensation to a point above the intensity of the permanent sensation. He refers to the researches of Berliner and others as having demonstrated that the sensation of red is more rapidly established and vanishes more quickly than the sensation of blue. Broca and Sulzer (9) state that the maximum of the curve of sensation is much more elevated for blue than for white of the same luminosity; much less elevated for the green; while the red is intermediate between green and white. For colored lights, as for white, the ratios of the sensation maxima to the permanent value are greater, the greater the luminosity.

As an auxiliary hypothesis, for the explanation of the colors of the Benham top, Pieron brings in diffusion or contrast. "When a small retinal surface, in the neighborhood of a region which is the seat of a luminous and chromatic process, is not or is only feebly excited, the excitation of this surface by diffusion carries with it a predominance of the chromatic process, which diffuses with a greater intensity than the luminous process." As I performed the experiments, the color changes were purposely studied in rather large luminous areas with the precise object of excluding, as far as possible, contrast phenomena. The succession of the colors was perfectly regular, and when the field was uniformly illuminated they appeared simultaneously over the whole field. With unequal intensity of illumination of different parts of the field the appearance of a given color was regularly advanced or retarded. Pieron gives the following figures for certain conditions of illumination: "from a time of illumination of 20 σ onwards, definite red coloration, then yellow after 40, green after 60, blue after 80." I gave in my paper an instance where the time of illumination which produced the red color was to that which produced the violet as 5 to 12; the times for red and green being as 7 to 12. In this particular experiment the duration of flash was about $\frac{1}{1200}$ second for the reddish phase, $\frac{1}{700}$ second for the green, and $\frac{1}{500}$ for the violet. In another experiment, in which a black and white picture of a landscape illuminated by an incandescent light was viewed through a disc with two opposite sectors of 45° cut out, the violet phase was obtained with $4\frac{1}{2}$ turns of the disc per second, the blue phase with 7 turns and the green phase with 10 turns per second. Here the duration of each luminous stimulus was $\frac{1}{38}$, $\frac{1}{56}$ and $\frac{1}{80}$ second respectively, and the number of stimuli per second 9.5, 14 and 20. Of course the choice of a point

at which a given color predominance is established is always more or less arbitrary, but a phase once determined can be reëstablished an indefinite number of times with considerable accuracy. The "rediscovery" after 30 or 40 years of facts of this kind and of their explanation, like "Benham's rediscovery" of the subjective color phenomena under discussion, is obviously based upon lack of acquaintance with the literature of the subject.

The explanations offered by Liveing and by Abney were little more than suggestions and were not worked out in detail. Yet Bidwell's summary rejection of them, on the ground that "if the thickness of the lines on the disk is much greater than 1 mm., or more accurately, if it subtends at the eye a greater angle than about ¼ of a degree, the red and some of the other colors appear only upon the borders of the lines, their inner portions remaining black or grey," is not justified, although his observation is correct. Apparently he was ignorant of my experiments showing conclusively that the essential phenomena can be observed and, as I think, better than with the top, under conditions which exclude contrast. Bidwell's observations on after images (3), (10) largely anticipated by Hess (2), of whose work he states he was ignorant at that time, afford no ground for rejecting the hypothesis that a difference in the time relations of the decay of the luminous impressions for the fundamental color sensations is a factor in the color phenomena produced by intermittent stimulation with white light, but rather the reverse. More striking changes than anything he has described, so far as I can judge, can easily be observed by viewing pictures or solid objects suitably illuminated by an ordinary electric lamp or by sunlight through a revolving disc. Some self-luminous objects show beautiful effects. The flames of a cannel coal fire become tinted in violet and green at a certain rate of intermission, the bright core of each tongue of flame being violet, the less luminous portion green. The phase is regularly changed according to the progression of colors already described when the rate of intermission is altered. Or with a constant rate of intermission the phase is changed by varying the intensity of illumination, as by approaching or withdrawing from the fire, narrowing or widening the aperture in the disc. Portions of a flame seen against a piece of black coal give the color phases (with a definite rate of intermission) appropriate to a less intense, and the free portions of the flame those appropriate to a more intense luminosity, and the same with parts of a flame seen through smoke. The essential phenomena can easily be seen without any apparatus, merely by viewing the fire through the narrow interval between two fingers when the fingers are approximated and extended and the slit thus formed moved more or less rapidly in front of the eye. The width of the slit can be varied at will by separating or approximating the fingers. Or a cylinder may be made by partly closing the fist and the object viewed through this, while a more or less rapid intermission is secured by

moving the hand. It is not even necessary to put a black glove on the hand. In this way the appropriate succession of tints can be easily seen in a black and white picture or in other objects which show them with the rotating disc, although usually not so sharply. They can also be observed when a cylinder of blackened paper of a diameter of 25 mm. or less is looked through while the cylinder, held between the fingers at the end next the eye, is moved rapidly to and fro so that the free end describes small arcs of a circle, interrupting the light while the eye continues to look through the proximal end.

When an object like a blazing fire, in which the light is itself more or less intermittent, is looked at without any interrupting arrangement, similar color phenomena may be seen from time to time, although of course less brilliantly and without the regular sequence. These have nothing to do with any objective coloration produced in the combustion. The same thing may sometimes be seen in flames generated on a larger scale. I first noticed such appearances (without intermission produced on purpose) in 1886 in flickering sunlight reflected from the wake of a Rhine steamer. Similar phenomena are sometimes seen from a moving train, in flickering lights (artificial or sunlight) through clouds of steam or the lattice work of a bridge or the intervals in a fence, etc. It is not probable that confusion as to signals could easily arise in this way, as here the subjective coloration is produced under quite unfavorable conditions. It is, however, perhaps not wholly unwarranted to raise the question. In some of the flickering electric street signs subjective color phenomena are easily observed.

The question suggests itself whether the color sensations of animals in an environment habitually or frequently illuminated by flickering light, as in forests or not far below water surfaces, could be modified at all by such phenomena as those under discussion. If so, protective color adaptation might in some degree be expected under these circumstances to take account of the factor of subjective coloration. Protective coloration has considerable interest in connection with the question of color vision in animals. For it seems in some cases to afford a means of testing whether the response in sensation to given wave-lengths is approximately the same as in man. Whether the subjective modifications of color sensation caused by intermission at appropriate rates enter at all as a factor into such phenomena as the aurora borealis, or some of the solar color phenomena, depends upon the magnitude of any intermittent changes of luminosity concerned in them and their period. Where the spectral characters of the light sufficiently explain the perceived colors there is of course no room left for a subjective factor.

It would be tedious to record the great variety of observations made by one or other of the methods mentioned, in all of which the general "march" of the color changes and their location on particular parts of the objects examined could be predicted from the theory of their production. When an expanse of snow illuminated by sunlight is viewed with the proper rate of intermittence it shows a violet tint at a certain rate. If a portion of the

snow-covered surface is in shadow this becomes deep violet, and with a further increase in speed blue. The greenish phase may or may not be reached according to the intensity of the illumination. A lawn which is brown in the spring before the grass has come on, or a clump of bushes still brown becomes distinctly green. Even the evergreen pine trees become greener with a certain speed of rotation of the disc when seen against the sky, with patches of violet where a grey or whitish sky is seen through the branches. Grey clouds become tinted violet and then bluish or even green, the subjective tint depending upon the original shade, the luminosity and the rate of intermission. The concrete pavements illuminated by sunlight appear violet at a certain rate of intermission, at a time when the mud on the street is showing a greenish tint and the puddles of water in the mud are violet or bluish.

A polyhedral object such as a white folded filter will show the green phase on the faces which are less illuminated at a time when the violet phase is being obtained on the faces which are better illuminated. When the rate of intermission is increased, with an appropriate strength of illumination, the violet changes to bluish and the green to yellowish green, and so on. A crumpled piece of paper will show with a certain speed green in the creases, where the illumination is less, and violet or blue where it is greater, and with increasing rate of intermission the appropriate color changes will occur. It can often be seen that stereoscopic effects accompany the color changes, the coloring of the shaded areas causing the relief to become more marked. This can sometimes be observed also in pictures, and by uninstructed persons, who say "the picture seems to be coming out to meet them."

An object like a porcelain dish will at a certain phase be violet in the interior where the luminosity is greatest and green of various shades on the edges and on the more feebly illuminated outside. A bundle of cotton wool teased out so as to admit light into its interior will show colors similarly corresponding to the degree of illumination and the rate of interruption. A strip of galvanized iron, coiled into a spiral and set so that the apex is nearer the light and therefore more strongly illuminated than the base, will have a violet apex and a green base at the same time. If the speed is now increased the apex will become bluish and the green will be seen to move progressively from the base toward the apex. A further increase in speed will make the apex appear green and the base may now be seen in its natural color. A florence flask filled with water set in front of an illuminated plate of ground glass, or simply lighted from above by a shaded electric bulb, shows the progression of the colors well when viewed through a rotating disc. The body of the flask can be made to assume tints like those of the bottles in a druggist's window, and the bands of light and shade in the neck are clothed in the colors appropriate for the

given rate of intermission and luminosity. A few drops of milk added to the water enhances the colors. Postage stamps, some bank notes, and other colored objects of this kind show similar changes corresponding to their objective coloration, when looked at intermittently. Matches can be made between the subjective colors and colored objects in the field whose tints are chosen so as not to be much affected subjectively. The most unpromising objects, such as dirty muslin rags or used blotting pads, undergo a remarkable transformation when viewed intermittently. A white hair comb will show violet teeth with green intervals with a certain rate of intermission, when seen by reflected light on a white ground, or by transmitted light against illuminated ground glass. An increase in speed will change the violet into bluish and then into green. The same object, seen by reflected light against a blackish background, will have green intervals and violet teeth, and when the speed is increased the teeth will become bluish and then green, the intervals showing red. A white feather shows the phenomena well. Objects such as lantern slides when viewed by transmitted light, for instance in front of an illuminated ground glass plate, demonstrate the colors excellently, the phase of the better illuminated parts being always more toward the violet than that of less luminous parts. Thus in a slide representing the surface of the brain, the sulci will be green at a time when the convolutions are violet or bluish, and every detail of the shading will be represented by the appropriate color nuance. Crystals, colored or colorless, deposited in a shallow glass dish and viewed either by transmitted or reflected light show the subjective color changes with remarkable brilliancy in appropriate cases. Colorless rings of greater or less opacity, such as can be obtained by permitting a liquid like milk to dry in a watch glass, appear in the expected colors when viewed intermittently either by transmitted or reflected light. The ghastly corpse-like or even gangrened appearance of a normal hand or of a rosy face (the eyes can be closed), when well illuminated and viewed by intermittent light is very striking, all the ruddy tints characteristic of life being enfeebled at the slower rates of intermission, and the violet and blue, at a somewhat greater rate the "cadaverous" greenish tints, coming out. The experiment recalls Victor Hugo's (12) description of the color of the windows of Notre-Dame, which "traversés d'un rayon de lune, n'avaient plus que les couleurs douteuses de la nuit, une espèce de violet, de blanc et de bleu, dont on ne retrouve la teinte que sur la face des morts."

When the colored (negative) after-image of an object is viewed against a white ground and the light from the white ground then intermitted, color changes appear in the after-image which do not seem to be essentially different from those seen when the same objective color is viewed. For example, the green after-image of a red sun just setting behind clouds was observed on a white paper. On turning the disc the first effect was

an extraordinary increase in distinctness of the after-image, the main central part of which, however, was now violet. With increasing speed this was surrounded by a blue border and outside of this a green border. When the speed was increased to eliminate flicker the after-image faded out, but reappeared on diminishing the speed. A similar appearance was seen with the after-image of an incandescent electric lamp, which was looked at for an instant and the gaze then directed to a sheet of white paper. The greenish tint of the after-image of the metallic filaments changed on slow rotation of the disk into violet, and the image became exceedingly bright. With a higher speed the image became blue and with a still higher green. On reducing the speed the intense violet color of the after-image of the filaments reappeared, and the experiment could be repeated as often as desired. It is often very striking to observe how the intermission of the light causes the after-image to appear in great intensity after it has been caused to disappear entirely by viewing the white surface with steady light. The color changes of the general surface of the paper as the rate of intermission is varied do not interfere with the study of those in the after-image of the filament, since the form of this remains perfectly distinctive. The phenomenon is easily seen without the revolving disk by viewing the white paper between the fingers and moving the hand. The fact that the after-image fades out when the speed of the disc is increased to the point of steady fusion, to reappear when the speed is reduced, suggests that its emergence is not due to a general lessening of fatigue by the intervals of relative darkness, since the ratio of the duration of illumination to that of darkness is the same for all speeds of rotation. Further the regular appearance of the color phases in the after-image with varying rates of intermission, just as when an external object is viewed, indicates that the process is fundamentally the same.

The appearances are, however, more complex and more difficult to follow since the after image is itself fading and changing color when viewed directly on the white ground.

For example, a spot of sunlight reflected by a mirror on to a white surface was viewed steadily, and the gaze then directed to a sheet of white paper illuminated by diffuse daylight. The after-image was at first yellow when viewed without rotating the disc. On slow rotation this changed to impure violet, and with faster rotation blue appeared in the centre with yellow outside. With a still faster rate of rotation the whole spot appeared yellow of the same hue as seen without intermittence. This was repeated many times. Later, when the after-image had faded so that it was not, or scarcely, visible with direct observation of the white ground, it was brought out distinctly yellow with fast rotation not far below the fusion point. The preliminary phases were given, though indistinctly, with the slow speeds, e.g. a dirty green, and before

that an impure violet or pinkish violet. With stronger sunlight the after-image was greenish without intermittence. On rotation this became violet mixed with blue in the centre; then with faster rotation, blue in the centre with green at the edges; then with still faster rotation the whole spot became green (brighter than with steady observation). A little later the after-image was orange or yellowish orange when viewed without intermittence, and this changed to violet with rotation of the disc.

When the after-image of the filament of an electric lamp was fresh and of a bluish-violet hue with steady observation on the white ground, slow rotation caused the lines to become brighter and to change to a green-yellow hue with purplish and scarlet borders. Later, when the after-image was greenish when viewed directly the lines became pinkish on slow rotation. When the after-image viewed steadily on the white ground was violet blue in the position of the filament and greenish in the interspaces, slow rotation caused the image to become pinkish. In another experiment the violet filament of the after-image changed on slow rotation to yellow. Later on, slow intermittence caused the violet filament with green interspaces of the after-image to become a pink filament with violet-blue interspaces.

I have devised a simple and very inexpensive arrangement which permits an unlimited number of interesting observations to be made by quite untrained persons, including children, on the subjective color phenomena under discussion. A common egg-beater (sold in two sizes for 10 and 25 cents respectively) is sawed through with a metal saw about 50 or 60 mm. from the cog-wheels. Of the two small cog-wheels only the one which gears directly into the large wheel is retained. A nut is fixed with solder or a small screw on the pin or wire on which it revolves near the small cog-wheel, so that it cannot slip in any position of the apparatus. The stumps of the two metal strips of the beater are sawed off at such a distance from the cog-wheel that when spread apart to a distance of 30 to 40 mm. at the free ends the pin projects 10 mm. or so beyond them. Blackened cardboard discs about 130 mm. in diameter with two, or two pairs, of opposite sectors cut out are easily prepared. It is not absolutely essential that the discs should be black, although, of course, it is better. The sectors are cut from the circumference to a depth of not more than 50 mm., leaving a ring of cardboard around the center intact. A small hole is made in the center with a cork-borer, and the end of the pin on which the small cog-wheel revolves passes through this. The discs are mounted so that the ends of the metal strips emerge at the bottom of two opposite open sectors, projecting a little beyond the surface of the disc, which is secured merely by passing a small rubber band around the two strips so as to clear the central pin. The discs can be mounted or dismounted in an instant, and

spin quite steadily when the larger wheel is set in motion by the small handle. When a disc with two deficient sectors is used the sectors can be conveniently made about 45° each, and about half this when there are four. Of course the speed of rotation necessary to obtain a given phase is about half as great with four interruptions for one turn of the disc as with two. By mounting two discs one over the other the breadth of the open sector can be varied at will. The little apparatus need not be fixed in a stand; it can be held in the left hand with sufficient steadiness while the wheel is turned with the right. A child can learn to do this in five minutes. The position of the rotating disc with reference to the object can be altered in any way desired, since it spins equally well whether the axis is vertical, horizontal or in an intermediate position. The observer can come nearer to, or go farther from, the object while he is observing it, and the object can be illuminated more or less strongly while it is being viewed, merely by placing it nearer to or farther from the light. The observations can be made in any room even in the daytime. The less diffuse light there is the better, but a dark room is unnecessary. It is sufficient to pull down the blinds. At night even this is not required. For examining pictures and many other objects by reflected light all that is necessary is to place them under an electric lamp such as can be obtained in most houses. It need not even be frosted. If there is much diffuse light in the room the lamp should be fairly strong, say a 40 watt metallic filament lamp, but it can be somewhat stronger without disadvantage and also somewhat weaker. There is of course no objection to a carbon filament lamp, if it is not too weak. A desk lamp is convenient, as it is already at a convenient height, or with an extension cord a lamp can be dropped to within a foot or so of a table. It should have an opaque shade so that the light is not directly seen. It is an advantage to cover the shade with a piece of dark colored cloth fixed with a safety pin. The picture can now be laid under the lamp either flat or at any angle. Its distance from the lamp can be easily altered by placing a few books under it. The best distance of the object from the lamp depends upon the strength of the latter and the shading of the object. Generally a picture should be pretty close to the lamp (60 or 70 mm.). The observer very soon learns how to bring out the various colors by increasing or diminishing the speed of rotation. In starting the disc he, of course, first sees that when it is at rest he views the picture through the deficient sector and not over the edge of the disc. Often it is advantageous to look at the picture from a considerable distance (6 to 8 feet or more). If the illumination of the picture is now diminished the most favorable distance will also be diminished. When the picture is viewed with both eyes, and one eye is now closed the color phase is advanced toward the red (parts which were violet or bluish, for instance becoming green), just as would have hap-

pened had the illumination been diminished by placing the picture farther from the light while it was still viewed with both eyes. Apparently the widening of the pupil of one eye when the other is closed does not make up for the diminution in the total effect caused by the elimination of one retina. Unglazed pictures, of course, are best, but it is possible to see the colors in glazed pictures also. The direct image of the lamp reflected from the glass must be kept out of the field, either by choosing a position where it is not visible or by covering a portion of the glass.

It is easy for several, or even a considerable number of persons to see the color phenomena in a picture or other object at one time by looking over each other's shoulders through the slit, or by using several "twirlers," or by regarding a mirror in which the picture is reflected when the disc is rotated between it and the mirror.

Pictures illuminated by sunlight can be viewed by drawing the blinds in such a way that a patch of sunlight falls on them as they lie on the floor or on a chair, while most of the light is shut out. Many interesting appearances can be obtained simply by viewing objects at any distance from the house through a window while the disc is rotated. It is not necessary to darken the room, but may often be advantageous to stand in a dark corner. Similar appearances are seen without the disc, although less conveniently and generally less sharply marked, by intermitting the light with the fingers, as previously described. This method or the disc can also be used in the open air under proper conditions.

For my own work, I have often used the small "twirler" described, but also in a dark room a larger fixed apparatus for color-mixing, carrying discs 230 mm. in diameter with an open sector which can be increased from 0° to 90°. Very "massive" color effects are obtained by viewing black and white pictures or other objects in this way.

Studies on color-blind persons with this convenient apparatus might yield results of interest, but have not hitherto been undertaken. A young professional man was apparently unable to see any color changes in some black and white pictures shown him although they were perfectly obvious to the other persons who looked at them. To him the pictures simply became "clearer" when looked at through the open sector with a speed of rotation which was bringing out marked green effects on the foliage to normal eyes. He stated afterwards that he had been told by an artist that his perception of colors was abnormal.

Since so many writers have referred to the phenomena seen with Benham's top I shall describe the appearances seen by me on discs constructed according to descriptions of the top and rotated at various speeds. For the sake of simplicity only the color changes in the position of the outermost and innermost groups of arcs were studied. Besides direct observation, the lines were also viewed through a weak hand lens. This permits exact location of the colors without causing any disturbing

color effects. The phase of the color with a given speed of rotation may be slightly altered by the change of luminosity due to the lens, but this is not important as the sequence is unaltered. A good lens may also be used in examining details of pictures with the open-sector twirler. When the disc was rotated in the opposite direction to that of the hands of a watch and the outer rings observed, the following color phases were seen with progressively increasing speed; 1, a darkish green upon the lines, with violet or rose-violet in the interspaces; 2, purple or dark violet lines with greenish yellow interspaces; 3, red lines with rather vague colors in the interspaces (blue, yellow and green shades mixed); 4, violet on the lines, with bluish shades in the interspaces; 5, greenish lines (lighter than in the first phase) with greenish interspaces. These observations were made with a 40 watt metallic filament frosted lamp (well shaded) 330 mm. above the disc. Phase 1 was obtained with very slow rotation (1½ to 2 turns of the disc per second); phase 2, 3 turns; phase 3, 6 turns; phase 4, 10½ turns; phase 5, 18 turns per second.

With rotation in the direction of the watch hands and observation of the inner group of arcs with the same illumination the following colors were seen: The greenish first phase on the traces of the arcs could be seen, but on account of the small size of the circle could not be so distinctly followed as on the outer group. Phase 2 (purple or dark violet arcs with yellowish green splashed with some blue or violet in the interspaces) 3½ turns of the disc per second; phase 3, 6½ turns; phase 4, 10 turns; phase 5, 19 turns per second.

In another experiment the lamp was brought nearer the disc (distance 210 mm.) and only phase 3 (red lines) observed. It was obtained on the inner group of arcs with 7½ turns of the disc per second; and on the outer group (with rotation in the opposite direction) with 6½ turns per second. No attempt was made to get precisely the same shade of red as on the inner arcs.

The explanation of the phenomena offered is based on my observations with illuminated surfaces or portions of pictures where contrast effects are avoided: namely, that with the slowest rates of intermission violet (or blue) predominates, with an increased rate green, and with a still more rapid rate red, so that as the speed is progressively increased the predominant color phase passes always toward the red end.

An apparent difficulty is that on the arcs, with the direction of rotation which gives the red phase, the series seems to be repeated: green, (under proper conditions yellow), purple (red and violet mixed), red, constitute a series in which the color predominance would seem to have passed red-wards as far as it can go. But after phase 3 the sequence starts again and we get violet and then green. The difficulty is only apparent. Phases 4 and 5 alone belong to the series of color changes observed on relatively large surfaces. The earlier phases are due to the fact that owing to the presence of the black arcs a condition of the retina is created which permits the establishment of subjective color phases more easily than with an unbroken white surface and therefore the differences in predominance of the fundamental sensations come out with a slower rate of intermission there than elsewhere. This is evident from the rates of intermission given above. It is also clear from observing the disc that with those slow rates vivid color effects are already present on and between the lines but not elsewhere. The black lines protect the retina from stimulation for an angular movement of the disc of 45° before it is affected by the white light which has already been acting on the retinal areas corresponding to the image of the interspaces. The conditions for contrast are evidently thus created, and for the present purpose it is indifferent whether we consider that the bounding of the interspaces by unstimulated edges renders them more sensitive than the general surface of the disc, so that the emergence of the normal sequence of predominance of the fundamental

colors is, so to say, more precocious over the interspaces than on the general surface and that the traces of the arcs then show by contrast in the complementary colors, or whether we assume that in the position of the arcs the white light acting on a relatively unfatigued retina more easily develops the differential effect of the fundamental colors. In the latter case the predominant color phase would naturally be nearer the red end than in the interspaces because of the smaller average luminosity over the rings. Bidwell's statement that if the arcs are much more than a millimeter in thickness some of the colors (including red) are only seen at the edges is against the last assumption. This point will be returned to. In the meantime, let it be repeated that the first sequence of color changes (included in phases 1 to 3 as above described) from green to red on the traces of the arcs, from violet to blue, green and yellow in the interspaces, represents a rehearsal of the same general sequence afterwards to occur at higher rates of intermission upon the general surface, the precocity of the phenomena being due to the fact that the retina had been specially prepared to develop the subjective color predominances by reason of the existence of the black arcs. Next comes, with phase 4, the beginning of the sequence which is now invading the general surface, and this is continued in phase 5. That this is correct is indicated by the fact that when the violet of phase 4 or the green of phase 5 is being obtained, say on the outer group of arcs (with rotation against the clock-hands), approximately the same shades of violet and green are present on the arcs of the inner group, which of course have never shown phases 1 to 3 with this direction of rotation, and whose traces are not being directly followed by white but by the black half of the disc. With increasing speed of the disc the condition of the retinal area occupied with the images of the arcs and their interspaces may be expected to approximate more nearly to that occupied by the general surface as regards any differential response to the fundamental colors. At such rates of intermission as yield the fourth and succeeding phases the differences between the curves of rise and decay of the sensations may be greater than at lower speeds so that any residual differences in sensitiveness of a given portion of the retina to these effects may be less influential than at the lower speeds.

As regards the color phenomena seen on arcs with rotation in such a direction that black succeeds to the image of the arcs, all that need be said is that there is practically nothing to represent phases 1, 2 and 3, as described above. The colors seen in the intervals between the arcs are violet, blue and green successively as the speed is increased, as on the general white surface, but anticipating the general changes somewhat, particularly with the slowest speeds. Thus, in one experiment (with 40 watt lamp 330 mm. from the disc) the following comparisons were made between the outer group of arcs, giving all phases with rotation against the watch-hands, and the inner group. With the slowest speed the intervals between the inner group of arcs were faint violet, the arcs very dark violet. The arcs of the outer group were changing at this time from phase 1 to phase 2, showing yellow sometimes. With higher speed the intervals between the inner arcs were bluish violet, the arcs a darker bluish violet. At this time phase 3 was present in the outer group, the arcs showing red. With still higher speed the intervals of the inner group showed blue and green, and the arcs were changing from bluish violet to green. At this time the colors on the outer group were changing from phase 4 to phase 5 (green).

The lack of the earlier phases on the inner group is easily understood when it is remembered that after the impression of the arcs has passed over the retina, there succeeds a long interval of darkness during which with the slower speeds any retinal change due to the arcs is effaced or much weakened, so that the white light comes upon a part of the retina which does not differ much from the rest. The opposite state of affairs is found when the rotation is in the direction of the watch-hands.

Hence the color phenomena seen on, and between, the inner arcs with one direction are naturally seen on the outer ones when the rotation is reversed.

As the set of arcs makes its impression on the retina during an angular rotation of 45° and the plain white surface during an angular rotation of 135°, it was of interest to observe the colors given when the disc was at rest and, like the pictures, was viewed through the open sector of a revolving black disc. This is evidently a simpler experiment, as are the observations with pictures, than viewing the rotating disc with white and black sectors. For the same image is always thrown on the retina as the open sector comes round, and not a succession of different impressions as with the rotating disc. The illumination was that of the 40 watt lamp at a distance of 210 mm. There was, of course, practically no difference between the different groups of arcs by this method of examination. When a group was viewed with 10 interruptions a second through an open sector of 45° dark violet was seen on the arcs and yellowish in the intervals. A little increase of speed gave dark green on the lines and greenish in the intervals. On the general white surface of the disc violet was seen with 10 interruptions a second, violet with some blue at 13 interruptions; bluish at 15 interruptions; and greenish at 18 interruptions a second. When a color appeared on the general white field it could also be seen in the intervals between the arcs in any group, only more pronounced than on the general field; this was especially distinct in the case of the first violet which appeared. With increase in speed of the rotating black disc came blue, then greenish, and then yellow, all more marked between the arcs; only when green was reached this spread over the arcs as well, and the same with yellow.

With the black disc with an open sector of 135°, a violet mixed with blue was given over the general white surface when the rate of interruption was 7 times a second; with 10 times a second, blue mixed with some green and yellow. Another observation gave violet with 6 turns and blue mixed with some green with 10 turns of the black disc per second. The arcs on the white field in the disc observed were about ½ mm. thick and 2 mm. apart. When the greenish phase was obtained on the stationary disc viewed through the rotating open sector, the groups of arcs appeared greener than the general white field. When lines are drawn nearer to each other than this, as in a pen and ink drawing, the difference in the intensity of the green over the shaded area at a certain speed of interruption is marked. When the black lines are near enough the eye does not perceive any difference in subjective coloring of lines and intervals, and the effect of the shading is the same as that of a diminished luminosity.

Some observations were made on a disc with two groups of arcs, one in the position of the innermost and the other in that of the outermost groups of Benham's top. Four arcs were drawn in each group; some of these were thin (¼ to ½ mm.) and others thick (1 to 2 mm). With rotation of the disc in the direction of the watch-hands, the colors seen at different speeds on and between the outer group of arcs did not differ essentially from those seen on the disc with thin arcs already discussed. Nor was there any marked difference in the colors seen on the thick and thin arcs in the same group. In general these colors were similar to those in the interspaces and the general ground, differing merely to some extent in brightness. Only with the slowest speed, when the thin lines were showing a greyish violet the thick lines remained black with indistinct thin grey violet edges. It was different with rotation in the opposite direction. Here on the outermost group the same 5 color phases were seen on the thin arcs as already described. But on the thick arcs the first phase (green) was especially evident at the edges of the arcs, although spreading also inside them. With higher speed the thin arcs showed phase 2 (purple or dark violet), while the thick arcs were black with a slight dark purple tinge; but they did not show even as much color over their general surface as in phase 1. With higher speed (phase 3)

while the thin arcs were red, the edges only of the thick arcs showed a red color of about the same hue as that on the thin arcs. The general surface of the thick arcs was dark with a purplish or dark violet tinge. The interspaces had all about the same hue (bluish green). With increased speed (4th phase) an impure violet was seen on the broad arcs apparently extending over the whole breadth of the arcs. With a little higher speed the thin arcs assumed the same violet tint, but with a tendency for the red shade to persist till the speed was increased. With higher speed all the arcs became of about the same greenish hue, the general field being also greenish. For the last two phases there was no essential difference between the outer and inner group of arcs. With reversed rotation of the disc all the phenomena described for the outer group were naturally obtained with the inner group.

SUMMARY

Attention having been called to studies on subjective color phenomena caused by intermittent stimulation with white light published by the author in 1888, many unpublished data obtained then or subsequently are now presented. A simple method is described by which the colors can be produced in a striking manner, particularly in black and white pictures, by persons without training, including children. No laboratory or laboratory equipment is required. The small "twirler," which could probably be made for a few cents, is the only apparatus needed, except such an electric lamp as can be found in most houses.

Studies have been made on the color changes produced in colored after-images by intermitting the light from the ground on which they are viewed. The changes are essentially similar to those seen when external illuminated objects are viewed with a rate of intermission below that required for fusion, and occur in the same general sequence, although the phenomena are more complicated owing to the color effects associated with the decay of the after-images themselves and other factors.

An explanation of the color changes seen on rotating Benham's top, based on the writer's previous observations and conclusions, is offered.

BIBLIOGRAPHY

(1) STEWART: Proc. Roy. Soc. Edin., 1888, 445–464.
(2) HESS: Pflüger's Arch., 1891, xlix, 190.
(3) BIDWELL: Proc. Roy. Soc., 1894, lvi, 132.
(4) BIDWELL: Proc. Roy. Soc., 1897, lx, 368.
(5) LIVEING: Nature, 1895, li, 167.
(6) ABNEY: Nature, 1895, li, 292.
(7) Brit. Med. Journ., 1895, i, 272.
(8) PIERON: Compt. rend. Soc. de biol., 1922, lxxxvi, 922.
(9) BROCA AND SULZER: Journ. de physiol. et path. gén., 1904, vi, 55.
(10) BIDWELL: Proc. Roy. Soc. 1897, lxi, 268.
(11) HUGO: Notre Dame de Paris (Hachette et Cie, 1881, ii, 188).

XUM

SECONDARY EXCITATION IN THE RETINA

WILLIAM R. AMBERSON

From the Department of Physiology, University of Tennessee, and the Department of Physiology, University of Pennsylvania

Received for publication April 21, 1924

When, in a dark room, the right eye is directed toward a small spot or rectangle of red light, of low intensity, so that fixation is somewhat to the right of the illuminated area, there are seen, streaming out to the right, two bands of bluish light, which appear to arch out from top and bottom of the light source, and then converge toward each other in the direction of the blind spot. An illuminated vertical red slit window, 5 cm. high and 1 cm. wide, viewed at a distance of one meter, with fixation directed to a point 2 cm. to the right of the middle of the slit, gives a good demonstration of the effect. The form of the bands, viewed under such conditions, as seen in my own right eye, is represented in figure 1. The outer edges of the bands stand out fairly sharply against the dark background the inner borders are more vague. At times the whole enclosed field seems filled with a faint bluish haze.

In the left eye a similar pair of blue bands, streaming out to the left, is found. In both eyes the effect is transitory, fading out in about one second, to reappear when fixation is slightly shifted or the eye is winked. The bands can be elicited by any color of primary stimulus, becoming, however, progressively more indistinct as shorter wave lengths are used.

This curious entopic effect appears to have been first noticed by Purkinje (1). It was later reported by Charpentier (2), Zeeman (3), Tscherning (4) and Hubbard (5), none of whom were aware of the previous descriptions. Tscherning published rough sketches of the effect. Ten Siethoff (6), attracted by Zeeman's note, attempted a further study and explanation of the phenomenon. Hans Gertz (7), (8), (9) published a series of papers dealing with the effect. Otto Gertz (10), finding the descriptions of Purkinje and Tscherning, and unaware of Hans Gertz's lengthy theoretical treatment, again described the effect, and suggested an explanation which corresponds closely with that of Hans Gertz. Thomsen (11), in a recent review of Purkinje's entopic phenomena, discusses the effect briefly. Troland (12) has used it in a study of the nature of the nervous discharge along the retinal fibers. So far as I can determine, these papers constitute the literature to date.

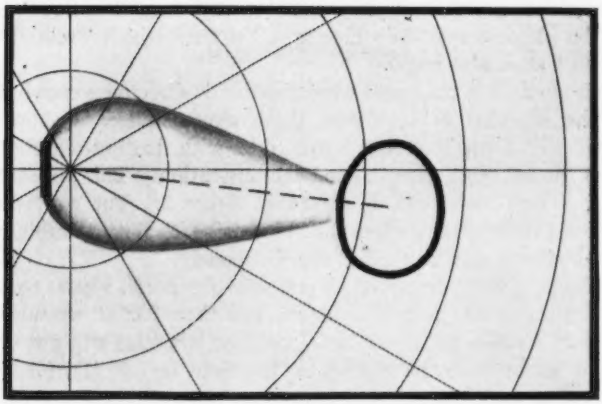


Fig. 1. The "blue-arcs" as seen by the right eye of the author. The right edge of the red slit lies one degree to the left of the fixation point, from which, as a center, arcs are described at intervals of five degrees of visual angle. The blind spot is outlined by the oval border. The papillo-foveal line is represented by the heavy broken line.

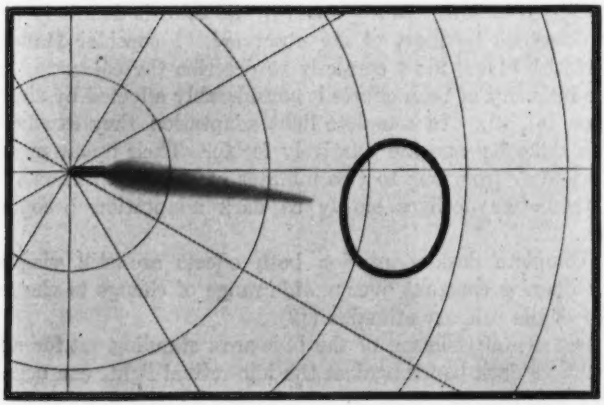


Fig. 2. The "blue-spike" as seen by the right eye of the author. Fixation is at the middle of the left edge of the red slit, which is placed horizontally.

Christine Ladd-Franklin has recently demonstrated the phenomenon at the Marine Biological Laboratory, Woods Hole, Mass., and elsewhere; it was through her that it first came to my own attention. She has named the phenomenon the "blue-arcs," a term which Troland adopts, and which I shall also employ.

A kindred effect is produced when fixation is directed somewhat to the left of the stimulus field. Under these circumstances a shorter horizontal red slit, 3 cm. long and 1 cm. wide, with fixation directed to the middle of the left edge, gives a good demonstration. This appearance, as seen in my own right eye, is shown in figure 2. For convenience in designation I shall call this effect the "blue-spike." With suitable stimulus fields both effects may be elicited simultaneously.

The following facts, described by previous observers, appear to be clear:

1. When the stimulus field subtends less than 0.6° of visual angle in width direct fixation of its center or right or left edge will not elicit the effects. Fixation must be slightly to the right or left (1), (4), (6), (8), (12).

2. The forms of the blue-arcs and of the blue-spike coincide very closely with the topography of the retinal fibers arising in the area of primary stimulation (2), (4), (5), (6), (8), (10), (12).

3. The color of the effects is variously described as blue (1), (4), (5), (9), (10), blue-violet (3) or violet (2), (6), (12). It is completely independent of the color and intensity of the primary stimulus. The saturation appears to be low. In my own eye the color is definitely blue, and it is so described by most of my observers. I consider Hans Gertz's term, "whitish-blue," most correctly to describe the color.

4. The intensity of both effects is considerably affected by the state of adaptation (8), (9). In complete light adaptation they can be secured only with difficulty and are relatively feeble. Their apparent intensity is greatest after from one to two minutes of dark-adaptation. The intensity thereafter declines slowly as dark adaptation becomes more complete.

5. In complete dark-adaptation both effects are still visible; their intensity remains constant over a wide range of change in the intensity and color of the primary stimulus (12).

6. A negative after-image of the blue-arcs, standing out for a fraction of a second as dark bands against the idio-retinal light, can occasionally be faintly seen (8), (9).

Purkinje was dubious as to the cause of the phenomena. He does state that the phenomenon is related to some normal organic structure within the eye, without identifying the structure involved. Charpentier suggested that some of the light from the primary stimulus, falling upon the temporal slope of the foveal concavity, undergoes reflection, being

thrown out nearly parallel to the retinal surface in the direction of the optic disc, finally to strike the curving retinal fibers, and illuminate them sufficiently to stimulate the receptors along their course. The observed absence of the effects, when the fovea alone is stimulated, renders this hypothesis untenable. Ten Seithoff believed the phenomenon to be an "entopic complementary after-image," produced by the stimulation of receptor cells in the macular region. He is not clear as to the mechanism of this stimulation. The constant blue color, with every color of primary stimulus, he thought to result from the selective macular absorption, because of which, he believes, more or less yellow light is always present in the neighborhood of the macula. Inasmuch as the effects appear almost, if not quite, coincidently with the viewing of the primary stimulus, and disappear at once with its removal, the time relations hardly indicate an after-image. Nor does the fact that the secondary effects extend far beyond the limits of the yellow pigmentation in the macular region seem to justify the second conclusion.

Hans Gertz (9), Hubbard (5), Otto Gertz (10) and Troland (12) agree in a more probable explanation of the effects. They conceive that the phenomena are both produced by a secondary excitation of retinal elements lying near the course of the retinal fibers which originate in the region of primary stimulation, and along which nervous discharges are passing. The electrical variations associated with such discharges are sufficiently strong to reach out and involve these other elements. Sensations rising to consciousness from these secondary elements are projected to that portion of the visual field which corresponds with the position in the retina of the elements involved. In this way we are actually able to see the course which the nervous discharges from the region of primary stimulation are taking through the retina.

With this general conception I am in full agreement. The evidence now to be described confirms the theory. In addition it contributes certain observations which bear upon the identification of the retinal elements which are secondarily excited. All of the above mentioned theories have considered the effects to be peripheral phenomena. There has remained, however, the possibility that they are more centrally produced, and that they might even be cortical events. My own observations point very clearly to the peripheral location of the effects.

Experimental. Although the effects are to be related to retinal structure, it is more convenient to speak in terms of the projected fields, since experimental values are so determined. Unless otherwise stated, this manner of speaking will be consistently used.

The retinal areas within which the primary stimulus must fall in order to elicit the secondary phenomena are quite limited in extent. I have explored these areas in my own right eye. In this study I have used a

stationary field, and a movable fixation point of luminous paint, which can be easily and quickly adjusted in various positions in relation to the stimulus field. In figure 3 the results of a typical experiment are shown. With stimulus fields of the sizes stated above the outlines of these areas are approximately as shown in full lines in this figure. With a smaller field, 2 cm. long and 4 mm. wide, the outlines are approximately as shown in broken lines. Those retinal elements which, when stimulated by a vertical field, can give rise to the blue arcs, appear to be confined to the areas to the left of fixation; those which, when stimulated by a horizontal field, can give rise to the blue-spike, appear to be confined to the areas to the right of fixation. I have drawn the outlines to indicate the probable

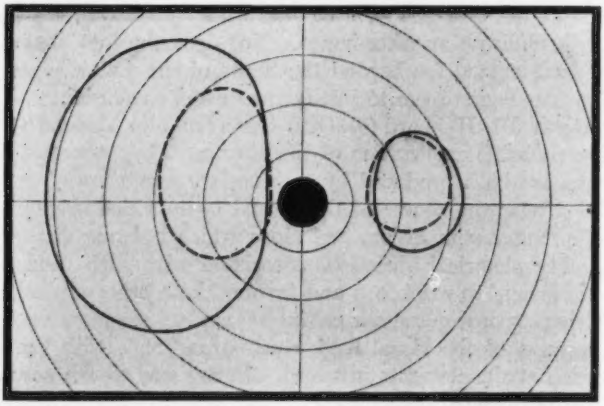


Fig. 3. The retinal areas (projected) within which the primary stimulus is effective in producing the secondary phenomena. The central black circular area represents the size and position of the fovea. The "blue-arcs" are produced when a vertical stimulus field excites the areas to the left; the "blue-spike" when a horizontal field excites the areas to the right. Full lines indicate areas as determined by large stimulus fields; broken lines indicate areas as determined by small stimulus fields (see text). Concentric circles are drawn at intervals of one degree of visual angle.

extent of the retinal elements which can be involved, rather than the field which would wholly include the stimulus fields in the various positions. For instance, the threshold distances from the fixation point, for the areas to the left, are determined by observing at what distance from fixation the left edge of the vertical field must lie in order that the first faint traces of the arcs can be obtained. Here presumably the field begins to invade that retinal region in which those elements lie which must be primarily stimulated in order to secure the secondary phenomena. Similar considerations are applied to the determination of other points on the borders of the areas.

The best demonstrations of the blue-arcs are obtained when the vertical stimulus fields lie wholly within the areas to the left, bisected by the horizontal line through fixation. The maximum intensity and length of the arcs is obtained when the right edge of the field is from 2 to 6 cm. to the left of the fixation point. On either side of these positions the arcs become fainter and fainter, and disappear at the limits of the areas. As the field comes to lie farther and farther to the left, within the area, the two arcs become progressively wider apart, and more curved, and the inner borders, originally vague, become as well defined as the outer. The bluish haze, filling the enclosed region, is seen only when the stimulus field lies close to the fixation point.

Any departure from this symmetrical arrangement of the field affects the intensity of one or the other arc. As the field comes to lie more and more above the horizontal line through fixation the lower arc becomes fainter and fainter, and finally vanishes; the upper arc maintains its intensity for a time, then also weakens and vanishes. The reverse is true when the field lies more and more below the horizontal line. By a proper choice of positions either arc may be obtained alone. The upper and lower limits of the areas plotted in figure 3 indicate the points where the last traces of one or the other arc can be secured. The size of the stimulus field is obviously an important factor, particularly in the areas to the left of fixation.

The central area of "blindness" for the secondary phenomena is a matter of some interest. The width of this region as determined from the data plotted in figure 3 is 2° 4′ of visual angle for the larger fields, and 1° 52′ for the smaller fields. This gives an average width of 1° 58′. This central area corresponds fairly well with the known size of the rod-free area. According to Parsons (13) this latter area subtends about 3° at the maximum, with considerable individual variation. The fovea, according to Parsons, subtends about 1°. Its size and position in relation to the areas previously discussed are shown in figure 3. It is seen that the secondary effects are absent when foveal cones alone are stimulated, and are also lacking beyond the fovea in a region whose total width is about that of the rod-free area. I have therefore been forced to the conclusion that the effects under discussion are rod, rather than cone, phenomena, in the sense that the primarily excited and conducting fibers must arise from ganglion cells on the rod pathways in the region of primary stimulation. So far as I can discover, this correlation has not been previously suggested.

If this conclusion is correct it may indicate that the rod-free region is not symmetrically disposed about the fovea, as is usually assumed, but that in the retina the fovea lies eccentrically, toward the temporal side of the region. In the light of this conclusion the invariant blue color of the

XUM

two effects, independent of the stimulus color, becomes somewhat more intelligible. For the rods react alike to all color stimuli, giving for all a grey or white sensation, and presumably there will be no qualitative change in the character of the nervous discharges along rod pathways with color changes in the primary stimulus.

The coincidence between the form of the two effects and the topography of the retinal fibers in the regions involved is truly striking. Vogt (14) has recently published excellent drawings of the distribution of the retinal fibers in the regions involved. It is seen that, in the neighborhood of the fovea the general radial arrangement of the retinal fibers is broken, as a result of the peculiar necessities of foveal vision. Fibers arising to the temporal side of the fovea are directed around, instead of through it, and so pursue their course to the nerve head in the form of an arc. Two systems of such fibers, after passing, the one above, the other below, the fovea, converge toward each other, running symmetrically with respect to the papillo-foveal line as an axis, and so reach the nerve head. Fibers arising from the fovea itself, and from other visual cells lying to its nasal side pursue a direct course to the nerve head.

In correspondence with these anatomical peculiarities the two effects show strikingly similar forms. In my own eye, and in many others which I have studied, the axis of symmetry for the two blue arcs is definitely inclined downward along the direction of the papillo-foveal line. The blue-spike is similarly inclined, making in my own eye an angle of about 6° with the horizontal as compared with an angle of 7° for the papillo-foveal line as determined independently with the perimeter. In one other eye which I have studied the blind spot is almost exactly bisected by the horizontal line, which is therefore also the papillo-foveal line. In this eye the blue-spike appears to stream out horizontally, and the axis of the blue arcs is also horizontal.

The opening out of the blue-arcs, as the stimulus field is moved farther and farther to the left of fixation as described above, accords exactly with the topography of the retinal fibers, since the arching course which they pursue becomes more and more bowed as we consider regions farther and farther to the temporal side of the fovea.

When the absolute length of the arcs is considered the coincidence with the retinal fibers is again most striking. In measuring the length of the arcs I have employed an iron upright, dimly illuminated by a coat of luminous paint. When the vertical stimulus field is viewed under the standard conditions described in the introduction the end of the arcs can be marked by moving this illuminated upright at right angles to the line of sight, at the distance of the stimulus (1 meter). The distance from fixation is determined by at least four independent settings, and the length of the arcs, in visual angle, determined by calculation from the

average of these readings. Although the coincidence between the length of the arcs and the distance to the blind spot has been mentioned by several of the previous observers, no quantitative data have been published. I therefore submit, in table 1, the results of determinations of the length of the blue arcs in ten normal eyes, including my own. In the parallel column are given the distances from fixation to the near edge of the blind spot, measured along the papillo-foveal line, as determined independently by perimeter reading. The averages of the two columns check very closely.

I have recently been able to secure further evidence of a different nature which confirms and extends the above observations. In one of my students, Mr. Beatus, I have been able to observe the effect upon the secondary phenomena produced by a paracentral scotoma lying between fovea

TABLE 1

Length of blue arcs compared with papillo-foveal distance

OBSERVER	NUMBER OF READINGS	LENGTH OF BLUE ARCS	PAPILLO-FOVEAL DISTANCE
D. E. G.	4	13° 46′	13° 2′
T. J. M.	4	12° 33′	13° 46′
H. H. K.	6	13° 32′	12° 34′
A. A. O.	4	12° 34′	12° 56′
L. N. H.	4	13° 56′	12° 4′
W. R. A.	4	13° 12′	13° 34′
G. D. A.	4	13° 56′	13° 48′
B. L.	6	14° 18′	13° 15′
M. McC.	6	11° 55′	12° 24′
G. M. G.	4	12° 56′	13° 0′
Average		13° 16′	13° 2′

and optic disc. This area of degeneration had resulted from a blow on the right eyeball, received in boyhood from a club in the hands of a playmate. The area was easily visible upon ophthalmoscopic examination, and could readily be mapped out by the perimeter. Foveal vision was still present, although the visual acuity was only one-third normal. In accurately locating the scotoma I have used a McHardy self-registering electric perimeter, reading in the dark room, and employing small stimulus fields illuminated by transmitted light. The instrument is furnished with a small illuminated fixation point and with an object carrier equipped with a white diffusing screen over which a series of colored filters can be drawn. I have read the values directly from the scale instead of using the self-registering device, thereby somewhat increasing the accuracy of the determinations. The form and length of the blue arcs were independently determined by a method similar to that described above.

In figure 4 the perimeter findings with white light are plotted, together with the average values for the blue arcs, obtained from a number of readings made at various times. The blue-arcs are quite abnormal. The lower arc is reduced to a remnant. Its end coincides closely with the left border of the scotoma. Once or twice the observer had the impression of a faint remnant of the outer end of this arc, but he could never certainly confirm this observation. The upper arc extends to and appears slightly to overlap the blind spot. It has a gap torn in it which corresponds well with the peninsula of degeneration extending upwards in this region. The coincidence between the form of the scotoma and these remnants of the blue-arcs is quite close—as close, certainly, as such measurements could be expected to give.

This coincidence seems clearly to indicate that the blue-arc phenomenon must depend upon the integrity of the retinal elements lying between fovea and blind spot. In this particular observer the vision of the left eye is normal; the blue arcs in it are also normal. The visual cortex is evidently normal. If the secondary phenomena were cortical events they should be normal even in the injured eye. Their abnormality in this eye and their close relationships with the form of the degenerated region makes the conclusion inescapable that the effects are retinal phenomena.

Surrounding the area of total blindness the perimeter revealed a much larger region of disturbance in color vision. In an extensive region light from a red filter (Wratten no. 70) was reported as white. In an even larger area light from a blue filter (Wratten no. 50) was seen as green. The outlines of this latter field are shown in figure 5. The scotoma shows only minor changes in form when read with the colored filters. With blue the blind spot appears somewhat larger than with white but the outlines cannot be so accurately determined. The larger size with blue is certainly in part due to the lower physical intensity of the stimulus, and to its lower visibility. The readings, with white, therefore, are more certain and in figure 5 the scotoma and blind spot are shown as in figure 4, as determined by white light.

Desiring a check upon these observations of a disturbed color vision I arranged to project a small spectrum into this area where blue appeared as green. The small region thus explored is represented as a shaded rectangle in figure 5. The subject reported that no blue at all could be seen in the spectral band, the band appearing uniformly green through the blue region; the violet could be distinguished with some difficulty as such. A slight movement of the prism brought the image beyond the affected region, and here the normal color sequence could be easily distinguished.

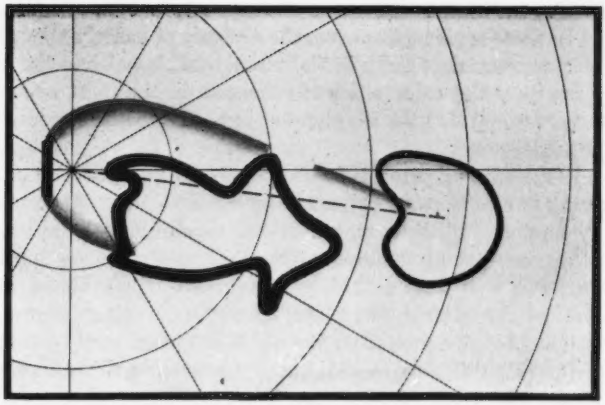


Fig. 4. The "blue-arcs" in the injured eye, superimposed upon the scotoma. Fixation as in figure 1. The blind spot, distorted in form, is outlined by the narrow border; the scotoma by the heavy border.

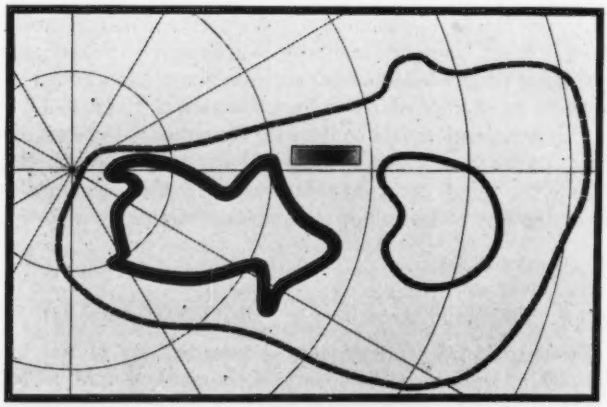


Fig. 5. The area within which blue is seen as green, outlined by narrow broken border. Blind spot and scotoma as in figure 4. The small shaded rectangle represents a spectrum projected upon a portion of the area of disturbance in color vision to check the findings with the perimeter.

We arrive therefore at the observation that in this eye a goodly portion of the retina, in which the remnants of the blue-arcs are produced, will not respond with blue sensations, when stimulated by blue light from without the eye. In these regions, however, the arcs are of exactly the same blue color which characterizes them in the normal retina. I had the observer carefully compare the color of these remnants in the right eye with the color of the normal arcs in his own left eye. He could detect no color difference whatsoever.

Troland (12) has suggested that the possibility of secondary excitation in the retina is considerably enhanced by the fact that the retinal fibers are unmyelinated. If the function of the myelin sheath be insulation, then its absence would facilitate spread of excitation to neighboring elements. It is well known that eyes are occasionally found in which small areas of the retinal fibers are myelinated. These areas usually extend out a short distance from the optic disc, but may be found at a distance from it. Wishing to determine whether, in a myelinated area, the secondary effects would be absent, I have endeavored to secure suitable subjects for the study. So far the Philadelphia clinics have not yielded an observer who was altogether suitable for the determination. I have recently studied the right eye of a subject in which there is an extensive retinal myelination. Unfortunately this area lies mostly to the temporal side, but a small region of myelination extends out from the disc toward the fovea. Ophthalmoscopic examination indicated that the retinal fibers which would produce the upper arc were normal unmyelinated pathways along their whole course, whereas the fibers which would produce the lower arc were myelinated for a distance of about 1° 40′ from the disc. If this myelination acts to insulate the primary nervous discharges preventing spread of excitation, the lower arc might be expected to be shorter than the upper by about this value. I therefore measured the two arcs separately. The values came out as follows:

Papillo-foveal distance	13° 24′
Length of upper arc	13° 11′
Length of lower arc	12° 3′

The difference between these two determinations of arc length is, however, within the experimental error of the method used, as an inspection of table 1 will show. The difference, therefore, may not be significant, and the evidence is entirely too meager to determine the answer to the problem. It does suggest that, in more favorable subjects, it may be possible to secure direct physiological evidence concerning the function of the myelin sheath, by a demonstration that in a myelinated region the secondary phenomena are absent. I shall hope to report more fully upon this matter when, and if, I can secure observers with a larger area

of myelination projecting toward the fovea. I wish to commend the problem to any others who may be more fortunate than myself in securing such observers.

DISCUSSION. The general conclusion appears justified that we are dealing with a case of secondary stimulation of retinal elements, lying along the course of the primarily excited and conducting fibers. When we attempt to determine the exact physiological mechanism of this secondary stimulation, and to identify the retinal elements secondarily involved, the problem becomes more difficult.

With regard to the mechanism of excitation, Hans Gertz (9) and Troland (12) agree in the view that the blue color is to be related to the colors obtained with electrical stimulation of the retina, as studied by Helmholtz (15), Müller (16) and others. These latter authors found that an electrical shock, applied through the eye in such a direction that the current (positive stream) passes outward along the line of sight, produces a flash of yellow or reddish-yellow light; applied in the opposite direction it gives a flash which is blue or blue-violet, of low saturation. Troland argues that "when the fibers connected with the retinal rods or cones of a small stimulated region of the retina are excited they suffer a so-called negative variation in their electrical potential. . . . . This . . . . would constitute a natural stimulus. . . . . If the electric current passes inward along the line of sight the color is a 'whitish-violet.' This latter color is the same as that of the blue arcs, and a moments consideration shows that the direction of the internally produced electrical current which we have assumed to be responsible for the generation of this phenomenon is also the same as in this case of the external application of electrical forces."

If Troland is thinking of the familiar electrical response of the eye, upon illumination, as he would appear to be, I am unable to agree with this argument. For the evidence of many observers (see 17) is quite conclusive that upon illumination the vertebrate eye suffers an electrical variation in such a direction that the cornea, or the fiber layer of the retina, becomes galvanometrically positive with respect to the receptor layer. At least the electrical change is in this direction, although the seat of its production may be questioned. Troland himself is quite aware of this fact for in an earlier paper (18) he specifically states that "the action of light is to augment the negativity of the receptor tissue." I am at a loss, therefore, to understand his argument in this instance. In any case it is quite certain that an externally applied electrical shock in the same direction as the internally produced electrical response of the eye, i.e., outward along the line of sight, elicits, not a blue, but a yellow flash of light. The electrical response of the eye itself, therefore, can hardly be invoked to explain the blue arc effect.

Other considerations militate against the conception. Fröhlich (19) has shown that, in the cephalopod eye, when a small circular area of the retina is stimulated, the electrical response is high within this area, but falls off rapidly to both sides in a symmetrical fashion, as more and more remote regions are considered. A similar situation undoubtedly exists in the vertebrate eye. If we could actually make contact directly with receptor cells and fiber layer we would probably discover that the constant electrical deflection observed in the eye upon local illumination is mainly, if not exclusively, generated in the region of stimulation. In any case any effect upon contiguous regions would be symmetrical in all directions. The entopic effects under discussion have absolutely no symmetrical relationships with the region of primary stimulation. The very exact correspondence between the forms of the two effects and the topography of the retinal fibers arising in the region of primary stimulation indicates that the secondary phenomena are in some way the result of physiological events occurring along these fiber pathways, and not in the region of primary excitation. We must have grave doubts as to whether the long-recognized electrical variation of the eye represents in any way the nervous discharges along the retinal fibers. It would appear very much more probable that the truth concerning these discharges will be found in the direction of Fröhlich's further findings on the cephalopod eye (19), (20). He reports a minute and rapid rhythmic electrical variation, superimposed upon the grosser constant variation which has long been known. This rhythmic variation is interpreted to represent the real electrical discharge along the visual pathways. More recently Chaffee, Bovie and Hampson (17), using a very refined technique, have secured curves which give indications of such rhythmic discharges in the vertebrate eye. These are, presumably, altogether similar to the well-known rhythmic discharges along efferent pathways, although of much lower voltage.

It is to such true currents of action that we must turn for an explanation of the secondary effects under discussion. In terms of the membrane theory, particularly as it has been given expression by R. S. Lillie (21) in this country, the electrical variation, or wave of negativity, implies a local self-propagating circuit completed through the medium external to the nerve. The work of Mayer (22) and Pond (23) has given excellent experimental support to the conception, which is indeed almost inescapable on purely theoretical grounds. Such external circuits, completed through the contiguous layers of the retina itself, give an easily intelligible mechanism for the secondary stimulation of other retinal elements. The same type of action is probably the basis of various irradiation phenomena and, indeed, of synaptic conduction itself.

Troland (12) believes that the rapid decline and disappearance of the secondary effects is due to the fact that the unidirectional electrical response of the eye, which he believes responsible for the secondary excitation, speedily reaches a constant maximal value, and thereafter, according to the DuBois-Reymond law, is ineffective as a stimulus. If the conception which I have been urging is accepted we must rather think of the transitory nature of the effects as being a fatigue phenomenon.

If the above conclusions are correct for the phenomena under discussion, we arrive at some basis for a judgment as to which retinal elements are secondarily involved. The current density in the external circuits is of course not the same for all retinal layers. There must be a relatively high current density in the zone immediately surrounding the nerve, and a rapid diminution in that density in zones lying more and more distant from the conducting fibers. We would therefore expect the retinal elements which are secondarily involved to lie close to these conducting fibers, since the stimulus strength is greatest in that region.

It has been suggested above that the primarily conducting fibers probably originate from ganglion-cells on rod pathways. If the preceding considerations are correct we might expect that the secondary elements would be contiguous nerve fibers arising as various points along the primary pathways, and running with them in the fiber layer. Both Gertz (9) and Troland (12) state that this cannot be the case. For, as Troland puts it, "among such fibers there are probably included some which connect with regions of the retina not lying along a continuous path between the stimulated area and the optic disc. The sensations derived from the secondary excitation will naturally be projected on that portion of the visual field which corresponds with the positions of the retinal receptors supplied by the nerve fibers in question, and the blue-arc effect is always confined to the region of the visual field between the primary sensation and the blind spot." For my own eye and for the majority of eyes this statement is correct. I find, however, among my students, young men twenty to twenty-four years of age, a number who report an extension of the blue field to the other side of the primary stimulus. I have found at least five such observers who, when observing the blue arcs with the right eye, often, though not invariably, see a distinct blue area to the left of the stimulus field, which appears to complete the ellipse outlined in part by the blue-arcs to the right. One of my observers is also quite certain that in the blue-spike effect he can occasionally see a distinct blue area to the left of the stimulus field, when fixation is directed considerably to the left of the field. These observations are perhaps not a certain demonstration of fiber stimulation, but they do suggest that in some eyes such direct stimulation of one fiber by another primarily excited fiber may occur. I am convinced that it may and does occasionally happen.

If the nerve fibers are not usually the elements secondarily involved, then the next most probable seat of the secondary action is the ganglion cell bodies, from which the fibers arise. The ganglion cells lie in immediate contact with the fiber layer and are hence in an optimum position for stimulation by the external circuits, generated in the primarily excited fibers. The current density around and through them must be very much greater than that to be found through any one of the deeper retinal layers. This consideration points very strongly to their identification as the elements which are secondarily stimulated.

Certainly the receptor cells are not the seat of the secondary effects. They are, in the first place, considerably removed from the conducting fibers (0.3 to 0.4 mm.) and are probably beyond the reach of the external circuits. In the second place the sensitivity of both rods and cones (24) is known to increase rapidly in dark adaptation, whereas the blue-arcs and blue-spike decrease in intensity with the development of this state. Thirdly, the evidence secured upon the pathological eye described above indicates that the receptors are not the elements secondarily involved. In a considerable area in which the cone cells are no longer capable of response with blue sensations when stimulated with blue or any other light, the blue arcs are present, unaltered in hue. Presumably, therefore, the cones are not involved. Nor is it reasonable to hold that an electrical stimulation of rods could produce the blue color since, when stimulated by any color of light, they are known to respond always with grey or white sensations.

There remains, of course, the possibility that bipolar cells are the seat of the secondary effects. Since they lie at a considerable distance (0.1 to 0.2 mm.) from the fiber layer they would appear to be much less advantageously placed for stimulation by the external circuits than the ganglion cells, and for the reasons already given I believe these latter to be a much more probable seat of the phenomena. I have therefore adopted, as a working hypothesis, the view that we are dealing with a secondary excitation of ganglion cells or of their fibers, effected by the external circuits of the rhythmic nervous discharges passing along nerve fibers which originate from ganglion cells on the rod pathways in the region of primary stimulation. It is not possible as yet to decide whether both rod and cone ganglion cells are thus secondarily involved. The definite blue color indicates that cone ganglion cells are certainly involved, but rod ganglion cells may also be stimulated. The low saturation of the secondary effects would rather point to a simultaneous involvement of the rod ganglion cells. The invariant blue color remains unexplained.

If the ganglion cells are the seat of the secondary excitation, then the decay in the secondary effects with increasing dark adaptation may be taken to indicate a process of "adaptation" in them, moderate in extent,

in the direction of decreased sensitivity. I know of no other evidence pointing to such functional changes in these cells. Such changes may very well be a factor of some importance in the development of dark adaptation, introducing a physiological process which may exert at least a moderate opposition to the increasing sensitivity of the receptors.

The evidence becomes fairly convincing, I believe, that in the production of these curious entopic effects we have a spread of excitation from one neurone to another without any synaptic connections between them. The above attempt at an identification of the retinal elements secondarily involved may be questioned, but there can be no question that the activity is communicated from fiber pathways utterly devoid of synaptic connections in the retina, to some other retinal elements. This general conclusion would seem to be a matter of some importance in the physiology of the nervous system. It may well occur in other situations, and be a factor in the production of many neural phenomena which are at present obscure. Particularly along unmyelinated pathways may we expect such secondary excitations. We are reminded of Ranson's (25) theory that unmyelinated fibers are the conductors of protopathic sensation. He believes that "insulation of the axon by the myelin sheath may play a part in eliminating the element of diffusion so characteristic of the more primitive sensations." The corollary of this conception is that normally a spread of excitation occurs along such afferent pathways with the secondary involvement of other unmyelinated fibers, not affected by the primary stimulus. The diffuse character of protopathic sensibility is thus explained.

SUMMARY

1. Two related entopic effects, the "blue-arc" and the "blue-spike" phenomena, produced by a primary stimulus affecting certain restricted regions of the retina, are discussed, and the previous descriptions summarized.

2. The restricted retinal areas within which the primary stimulus must fall, are described and a possible physiological test for the rod-free area discussed.

3. Measurements of the length and form of these effects, in the normal eye, are presented, and the close coincidence between them and the topography of the retinal fibers is confirmed.

4. Measurements are given of the blue-arcs in a pathological eye, in which a paracentral scotoma lies across a portion of that retinal zone in which the effects appear; i.e., between fovea and optic disc. In this eye the blue-arcs are mutilated, showing gaps which correspond closely with the outlines of the scotoma. The effects are therefore peripherally pro-

duced, and depend upon the integrity of retinal elements lying between the area of primary stimulation and the disc, being caused, presumably, by a secondary excitation of these intervening elements.

5. The mechanism of this secondary excitation is discussed.

6. Further evidence is presented to show that the effects are not due to secondary involvement of receptor cells.

7. It is concluded that the evidence indicates that the effects are due to a secondary stimulation of ganglion cells, or occasionally of their fibers, by the external circuits of rhythmic nervous discharges passing along retinal fibers which originate from ganglion cells on the rod pathways in the region of primary stimulation. This appears to constitute a demonstration that one nerve cell may communicate its activity to another nerve cell by other ways than through a synaptic junction.

BIBLIOGRAPHY

(1) PURKINJE: Beobactungen und Versuche zur Physiologie der Sinne, 1825, ii, 74.
(2) CHARPENTIER: Compt. Rend. d. l. Soc. d. Biol., 1885, viii, Ser. 2, 765.
(3) ZEEMAN: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorg., 1894, vi, 233.
(4) TSCHERNING: Klin. Monatsbl. f. Augenheilk., 1898, xxxvi, 223.
(5) HUBBARD: Psychol. Bull., 1910, vii, 196.
(6) TEN SIETHOFF: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorg., 1897, xiv, 375.
(7) GERTZ: Zentralbl. f. Physiol., 1905, xix, 229.
(8) GERTZ: Skand. Arch. f. Physiol., 1907, xix, 381.
(9) GERTZ: Skand. Arch. f. Physiol., 1909, xxi, 315.
(10) GERTZ: Hosp.-Tid., Køhenh., 1914, v, R., 7, 1197.
(11) THOMSEN: Skand. Arch. f. Physiol., 1918, xxxvii, 1.
(12) TROLAND: Journ. Opt. Soc. Amer., 1920, iv, 161.
(13) PARSONS: Introduction to the study of colour vision, 1915.
(14) VOGT: Handb. d. Biol. Arbeitsmet., 1922, Lief. 55, 376.
(15) HELMHOLTZ: Handb. der Physiol. Optik, 3te Auff., 1911, ii, 13.
(16) MÜLLER: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorg., 1897, xiv, 329.
(17) CHAFFEE, BOVIE AND HAMPSON: Journ. Opt. Soc. Amer., 1923, vii, 1.
(18) TROLAND: Journ. Opt. Soc. Amer., 1917, i, 3.
(19) FRÖHLICH: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorg., 1914, Abt. 2, xlviii, 354.
(20) FRÖHLICH: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorg., 1914, Abt. 2, xlviii, 28.
(21) LILLIE: Physiol. Rev., 1922, ii, 1.
(22) MAYER: This Journal, 1917, xlii, 469; xliv, 591.
(23) POND: Journ. Gen. Physiol., 1921, iii, 807.
(24) HECHT: Journ. Gen. Physiol., 1921, iv, 113.
(25) RANSON: Brain, 1915, xxxviii, 381.

STUDIES ON THE PHYSIOLOGY OF THE LIVER

VIII. Effect of Total Removal of the Liver on the Formation of Urea

JESSE L. BOLLMAN, FRANK C. MANN AND THOMAS B. MAGATH

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, and the Section on Clinical Laboratories, Mayo Clinic, Rochester, Minnesota

Received for publication April 22, 1924

It has been said (20) that too much valuable time and space have been given to the question of the function of the liver in the production of urea in the body, but the number of discussions on this subject attests to the incompleteness of our knowledge concerning it, as well as to the difficulties encountered in its solution.

A technic has been described (46) in which total removal of the liver from dogs allows the animals to remain normal, to all outward appearances, for as long as twenty-four hours afterward. In a previous report (47) we described the decrease in the amount of urea secreted in the urine, and the decrease in the amount of urea in the blood of hepatectomized animals. More extensive studies have made it desirable to determine the quantitative relationship between the amount of urea excreted following hepatectomy and the decrease in the urea content of the blood and tissues. These studies yield definite information regarding the site of the formation of urea.

Previous work on the relation of the liver to urea formation. The importance of urea in nitrogenous metabolism was first established by Roulle (70) in 1773, with the discovery of urea in the urine. Prevost and Dumas in 1823 isolated urea from the blood after extirpation of the kidneys, and numerous writers (83), (48), (49), (2), (65), (54), (22) have shown that the urea content of the blood and tissues is independent of the kidneys, which are merely the excretory organs for that substance.

The problem with regard to the site and the nature of the transformation of nitrogenous metabolic products to urea has been the subject of vast numbers of reports. Such problems may be divided into three opposing groups: those which indicate that urea is formed only by the liver; those which indicate that urea is formed by all the tissues, but chiefly by the liver, and those which deny that the liver has any special rôle in the formation of urea. Although a few (84) adhere to the first, the consensus

XUM

of opinion now, as judged by the standard textbooks of physiology (1), (35), (45), (88), and physiological chemistry (13), (20), (27), (52), seems to be in favor of either the second or third of these.

The methods used by the different investigators to determine the source of urea formation have been varied, and diametrically opposite results have been obtained with each method by different investigators. The older, chemical methods for the quantitative estimation of urea doubtless were sources of error, and even the present-day methods are not perfect. In many cases the physiologic methods used may be criticised even more severely than the chemical. The different methods will be grouped from a physiologic standpoint for discussion.

Comparisons of urea content of arterial and venous blood. Poiseuille and Gobley were the first to compare the urea content of arterial with venous blood. Using their own method for the determination of urea, they found that the venous blood to a certain organ sometimes contained more, and sometimes less urea than the arterial blood. Gscheidlen concluded that there was little difference between the urea content of arterial and venous blood, even of that passing through the liver. At first Picard (63) found similar amounts in both artery and vein, but later (64) more urea in the vein than in the corresponding artery. Grehant and Quinquaud found that the arterial and venous blood from the head or extremities contained the same amount of urea, but that the venous blood from the liver or spleen was richer in urea than the arterial blood. They concluded that urea was formed by the abdominal organs. Kaufmann observed no difference between the urea content of arterial and venous blood in the dog and horse.

Taylor and Lewis removed all the abdominal viscera, except the liver, from dogs, leaving the arterial supply to the liver intact. By comparing the urea content of the peripheral blood with that of the hepatic vein, they found no evidence of the liver having any special function in the formation of urea.

The futility of methods which attempt to determine the site of urea formation by comparison of the urea content of the blood flowing to an organ or tissue with the urea content of the blood flowing from the same tissue is readily seen by a consideration of the total amount of urea produced in relation to the blood flow from the organ or tissue. If the amount of urea produced at any given time is compared with the blood flow at the same time, it is evident that the present chemical methods are inadequate to determine the change in urea concentration.

Methods based on comparison of urea content of the tissues. Heynsius found urea in the normal mammalian liver in considerable amounts; Stokvis confirmed this, and added that urea was probably formed from uric acid in the mammalian liver. Meissner (55) found uric acid in the

liver of birds, and urea in the liver of mammals. He concluded that urea was formed in the liver from the products of the disintegration of blood cells destroyed in the liver.

Gscheidlen found the urea content of the different organs of the dog to be about the same as that of the blood, and concluded that the liver was not the sole source of urea. His findings were confirmed by Munk who used different and better methods for the determination of urea. Pekelharing directed attention to errors in Munk's methods, but with Hoppe-Seyler found that little urea was formed in the liver after death, and was unable to find either leucin or tyrosin in the normal liver or blood. They therefore concluded that these substances were not precursors of urea. Kaufmann found that the blood contained less urea than the tissues, but the liver and spleen more than the muscles. Schöndorff discovered that the urea content of the blood and tissues was about the same, the urea content of the muscles being slightly lower. Herter determined the urea content of the liver to be twice that of the muscles or brain.

Folin and Denis (15), studying the absorption and distribution of various nitrogenous substances, found that the urea content of the muscles was slightly higher than the urea content of the blood. They proved that urea, amino-acids, creatinin and so forth, were absorbed by the various tissues. Using cats in which the blood vessels to the kidneys had been ligated, they found that, following absorption of amino-acids in the muscles, the urea content of the muscles increased, whereas the non-protein nitrogen decreased.

Van Slyke and Meyer confirmed Folin's results, but found that the liver absorbed more amino-acids and liberated them sooner than the muscles. The urea content of the blood increased with the decrease in amino-acids in the liver; accordingly these authors, differing from Folin, concluded that the liver was especially responsible for the formation of urea.

Marshall and Davis, using Marshall's method of urea determination, found that the urea content of the tissues was approximately that of the blood, and that the injection of large amounts of urea increased the urea content in about equal proportion, except for the lesser amounts in fatty tissues and the larger amounts in tissues in contact with the urine.

Cathcart found that ammonia and glycocol injections in dogs increased the urea content of the tissues most in the liver and somewhat in the muscles. She concluded that urea was formed from these substances both in the liver and in the muscles.

There is no doubt that urea is widely distributed and with fair uniformity throughout the body, and probably all bodily secretions contain it in amounts comparable to that in the blood. The equal distribution of

urea is so constant that Hench and Aldrich have been able to estimate the urea in the blood by the determination of the urea in the saliva. Due to its solubility and diffusibility, it is rapidly absorbed by all the tissues. For this reason any conclusions with regard to the site of formation of urea, drawn from tissue analysis, must be open to question.

Methods using artificial perfusion and autolysis. Cyon found that perfusion of liquid through the liver enriched the fluid in urea. Gscheidlen showed this to be a mechanical washing out of the urea in the liver. Von Knieriem found that ammonium salts were converted into urea in the body, and the details of the building of urea by combination of ammonia and carbonates was shown by Schmiedebergh, who used Ludwig's method of perfusing the liver. Von Schröder (75), using ammonium carbonate in his perfusate, demonstrated the conversion of this substance into urea in the liver. He was unable to obtain any formation of urea by kidneys or muscles, and concluded that the formation of urea, especially from ammonium carbonate, was a function of the liver alone. Salaskin (71), using glycol, leucin and asparagin in perfusion of the liver, obtained a transformation of these substances into urea. This was also accomplished with many other amino-acids by Embden, Salomon and Schmidt. Fiske and Karsner (9) found that ammonia was readily converted into urea in the surviving liver, but that glycocol was not changed. They also found (10) that the liver poisoned by various toxic agents retained its power of converting ammonia into urea. Jansen (36), however, found that the perfused liver did form urea from glycocol, and concluded that the liver played an important part in the formation of urea from amino-acids. He further showed (37) that urea was produced even though the perfusate was already rich in urea, and concluded that this was not a reversible process in the liver. Löffler found leucin, alanin, glycin, aspartic acid and serin converted to urea by the perfused liver. Tyrosin, cystin and taurin were not so changed. Felix and Tomita found arginin converted to urea by the liver of the cat.

Kossel and Dakin (41) found arginase in large amounts in the liver of mammals, none in the muscles, and only small amounts in the kidneys and lymph glands. Fosse and Rouchelman found the urea content of the autolyzed liver (dog) to be greatly increased over that of the autolyzed heated liver. Hoagland and Mansfield incubated muscle aseptically for several days and found no increase in urea content. Levene and Meyer likewise found no diaminization of amino-acids incubated with leukocytes or kidney tissue. Hammett found that incubation of pulped placental tissue increased the urea content of the digesting mixture.

Methods of artificial perfusion promise much in the determination of the function of organs, but the contradictory results thus far obtained serve as an indication of the numerous technical difficulties encountered.

Methods based on removal of the liver. Nebelthau was unable to find any urea in the urine secreted after removing the livers of frogs, although he did find a marked increase in the ammonia excreted. Moleschott found urea still present in the muscles of frogs after the liver had been removed several days. Poulsson, after removing the livers of frogs and injecting ammonium salts in the lymph sacs, found that the urine contained considerable quantities of urea.

Städeler and Frerichs found the various tissues of sharks to be exceedingly rich in urea. Von Schröder (76) extirpated the livers of sharks and found that the high urea content of the muscles was unaffected. He attributed this to the failure of the kidneys to secrete urine, rather than ing to the removal of the liver.

Minkowski removed the livers from geese and studied the nitrogen elimination extensively. A marked decrease in the uric acid excretion and an increase in ammonia excretion resulted. The urea excretion decreased following hepatectomy, and it was not increased by injections of large amounts of amino-acids, although the ammonia excretion was increased. Urea injected subcutaneously was excreted unchanged.

Von Schröder (76) showed the formation of urea from ammonium carbonate in nephrectomized dogs. He closed the blood supply to the liver by ligating the hepatic artery and the hepatic veins, and anastomosed the portal vein to the left renal vein and extirpated the kidneys. His animals lived about two hours, during which time no increase in urea in the blood was observed, even after injection of ammonium salts; in some cases there was a decrease in the urea content of the blood.

Slosse ligated the celiac and both mesenteric arteries, which caused considerable decrease in the amount of urea excreted by dogs surviving for from four to fourteen hours after operation. He concluded that the abdominal organs exercised considerable influence on the formation of urea. Hahn, Massen, Nencki and Pawlow ligated the hepatic artery in dogs subjected to Eck's fistula. The urine of these animals contained increased amounts of ammonia, and a decrease in the urea excretion was observed.

Lieblein, using Pick's method of removing the liver (injection of sulphuric acid into the bile ducts), found that the urine of dogs so treated contained large amounts of uric acid. The urea excretion was little altered, but the ammonia excretion was markedly increased.

Kaufmann ligated the aorta and vena cava in the thorax (method of Bock, Hoffman and Seegan), and kept dogs alive for from an hour to an hour and a half by artificial respiration. He obtained slight increases in the urea content of the blood in some cases, but not in others.

Doyon and Dufourt ligated the hepatic artery and portal vein after partial removal of the liver, with the result that the excretion of urea was

XUM

markedly lower in relation to the total nitrogen excreted. Damage to the liver resulted in a decrease, but not in complete cessation, of the formation of urea. The ammonia excretion was relatively increased.

Salaskin (72) ligated the hepatic artery in dogs with Eck's fistula; the urea excretion decreased with the increase in ammonia in the urine. Biedl and Winterberg observed that animals with Eck's fistula, during meat intoxication, secreted increased amounts of ammonia and excreted decreased amounts of urea. They further found that injected ammonia was excreted as urea to some extent after ligation of the hepatic artery in these animals. Horodynski, Salaskin and Zaleski confirmed Biedl's results, but their conclusion differed in that they believed the liver to be entirely responsible for the formation of urea from ammonia.

Fiske and Sumner excluded the liver in cats by ligation of the celiac and mesenteric arteries, then the portal vein and hepatic artery, and after ligation of the kidneys found an increase in urea in the blood and muscle. After the injection of amino-acids these animals showed increases in urea content of the muscles and blood. The increase was not marked, but observations extended over a short period only (three hours) during which the animal was kept alive by artificial respiration.

Matthews and Nelson injected amino-acid mixtures intramuscularly into dogs with Eck's fistula after ligation of the hepatic and mesenteric arteries. Maintaining diuresis by injection of considerable amounts of diuretic fluids, they obtained a slight increase in the amount of urea excreted over that excreted by the control animals with no amino-acid injection. They obtained similar results by removing the entire abdominal viscera, leaving from 20 to 25 grams of liver tissue adherent to the vena cava. The amount of urea excreted decreased markedly in all of their experiments after the liver was removed, and in some cases they obtained a slight rise again after injection of the amino-acid mixture.

Perroncito removed most of the liver from dogs with Eck's fistula, leaving only traces attached to the vena cava. His animals recovered and seemed normal for a few hours. He noticed a diminution in the amount of urea excreted, and also a fall in blood urea, except in one case in which a piece of liver had been allowed to remain free in the peritoneum.

Many of these studies have been made with special reference to the relation of ammonia to urea. Folin and Denis (16) demonstrated the relative unimportance of ammonia, which was formed in the intestine and absorbed into the portal vein. Nash and Benedict almost eliminated ammonia from consideration when they demonstrated the formation of ammonia to be a function of the kidney. Recent experiments by Rich indicate that the methods employed for removal of the liver by ligation of its main blood supply are ineffective in completely obliterating the liver. The results of experiments when the liver was not entirely re-

moved should not be interpreted as meaning complete suppression of the function of that organ.

Clinical studies of hepatic disease. Frerichs, studying acute yellow atrophy, attributed the appearance of leucin and tyrosin in the urine to a cessation of the function of the liver in the formation of urea, and thought the nervous phenomena accompanying this disease due to the cessation of protein metabolism with the accumulation of toxic products in the blood. Winterberg and Münzer studied the results of urinalysis in many diseases of the liver: acute yellow atrophy, infectious jaundice, atrophic and hypertrophic cirrhosis, and found little evidence to indicate that the liver was the sole site of urea formation.

Frey gives an extensive review of the literature, and includes his own clinical data on urea and ammonia excretion in diseases of the liver.

Stadie and Van Slyke report a case of acute yellow atrophy in which the urine showed a low urea content with a high ammonia and amino-acid content. The blood contained a low value for urea, and the amino-acid content was high. Gilbert found high values of urea in the blood of patients with extensive blood destruction and jaundice. Both concluded that the liver was responsible for the production of urea, as in the atrophy the function was diminished and the urea formation decreased, while in the anemia the function was increased by the blood destruction, and the urea production increased.

Folin and Berglund found that ingestion of amino-acids in normal human subjects was followed by an increase of the amino-acid content of the blood, and an increased excretion of amino-acids in the urine. The urea content of the blood did not increase until the amino-acid content had begun to fall, and the excretion of amino-acids had returned to normal. The authors consider this to be evidence that the liver has no special function in the formation of urea, but that urea is formed by all the tissues.

Conclusions based on studies of extensive hepatic disease indicate that the liver has marked influence on the production of urea, but no definite conclusions seem possible. The extent of the lesions and the functional capacity of the liver may vary extensively, and the influence on other tissues is always to be considered.

METHODS. Since the method of removal of the liver has been fully described (46), only a brief description will be given here. The liver is removed in three stages. All operations are performed under ether anesthesia with aseptic technic.

The first stage consists in a reverse Eck fistula: that is, lateral anastomosis of the portal vein and the vena cava, and ligation of the latter on the cephalic side of the stoma. At first a considerable portion of the blood from the posterior portion of the body passes through the liver,

but since the capillaries of the liver offer more resistance to the flow of blood than is necessary for the development of collaterals through the azygos and internal mammary veins, most of the blood soon passes by way of the latter channels.

The second stage consists in the ligation of the portal vein at its entrance into the liver. This causes all the blood from the viscera and hind legs to return by way of the collateral vessels, the azygos and the internal mammary veins.

The third stage consists in removing the entire liver. This is accomplished by removal of the liver intact with the portion of the vena cava which it surrounds, so that no trace of liver tissue remains in the animal.

Recovery from the anesthesia and the immediate effects of the operation is rapid. Within an hour the animal is, to all appearances, normal; it walks around, responds to call, and exhibits the usual interest in its surroundings.

Three series of experiments have been performed. In one large series of experiments the liver only was removed. Some of the hepatectomized animals were allowed to continue their course uninterrupted until the development of symptoms and coma from hypoglycemia. This period varied from two to ten hours, after which some of the animals were maintained in a normal condition for several hours by the administration of glucose (46) sufficient to maintain a normal level of blood sugar. Others were given minimal amounts of glucose, so that they recovered from symptoms and merged into coma again; this process was repeated and the animal remained with a low blood sugar for a number of hours. Other animals were given glucose intravenously, by stomach tube, by jejunostomy, rectally, or by a combination of these methods, so that a normal or hyperglycemic level of blood sugar was maintained and the animals appeared normal for many hours. Solutions of amino-acids were injected intravenously into some of the animals of this series. Most of them secreted urine during the entire course of the experiment, and a few became anuric, shortly after the liver was removed, the volume of urine secreted depending much on the amount of fluids administered to the animals.

In another series of experiments both kidneys were removed from the animal at the time the liver was removed. These animals were maintained in a normal condition by the administration of glucose for about twelve hours.

In another series of experiments both kidneys were removed, eight and twenty-four hours before the liver was removed. The animals died from three to eight hours after hepatectomy.

For control animals for the first series many dogs were used, under identical conditions as regards diet and so forth. These dogs had been subjected to various surgical procedures under ether anesthesia for periods longer than that necessary for removal of the liver. For the control of experiments in the second and third series, animals under identical conditions were nephrectomized and treated similarly to the animals that were hepatectomized.

Specimens of blood were obtained with a dry syringe at varying intervals from the jugular vein and mixed with powdered oxalate in dry tubes. Specimens of urine were collected in metabolism cages, or by catheterization and washing the bladder, and by recovery of the urine in the bladder at necropsy. If the urine was desired at definite intervals. the animal was catheterized at stated times.

The following methods were employed for the quantitative determination of the nitrogenous constituents in the blood. Urea was determined by the Van Slyke-Cullen (86), (50) and Folin (12) methods, uric acid by

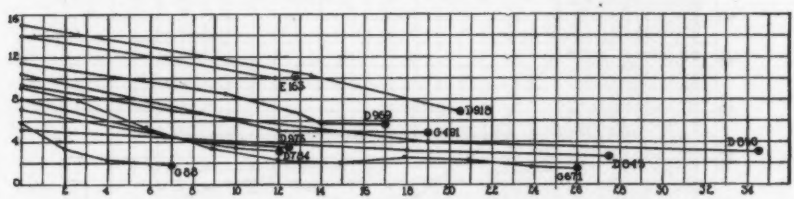


Fig. 1. Curves showing the decrease in blood urea nitrogen following removal of the liver. Urine was excreted at approximately normal rates for several hours after operation.

the Folin-Wu (12) method, amino-acid nitrogen by the method of Van Slyke (85), creatinin and creatinin plus creatin by the method of Folin (12), ammonia by the method of Nash and Benedict, and total non-protein nitrogen by the method of Folin and Wu (12).

Determination of the nitrogen in the urine was made by the method of Folin and Denis (12), or the Kjehldahl process. Urea was determined by the method of Van Slyke and Cullen (86), and Marshall (50), ammonia by the Folin-Farmer (12) procedure, uric acid by the method of Folin and Denis (12), and creatinin by the method of Folin-Jaffee (12). Amino-acids were determined by Van Slyke's (85) method.

Results. Urea estimations have been made on blood and urine in more than seventy-five hepatectomized dogs that lived from six to thirty-four and one-half hours after the liver had been removed. In every case in which urinary secretion was maintained there was a marked decrease in the urea content of the blood. Moreover, the amount of urea excreted in the urine showed a marked relative and absolute progressive decrease,

so that after a few hours there were only minimal amounts of blood and urine. In animals that became anuric, no further decrease was found in the urea content of the blood, and in no case was any increase noted which was not within the limits of experimental error of the methods employed. The animals that became anuric at the time the liver was removed showed no change in the urea content of the blood. This decrease in blood and urine urea was unaffected whether the animals were allowed to become hypoglycemic for long periods, or glucose was administered either in minimal or maximal quantities. The administration of glucose and fluid had no effect other than its effect on diuresis, except

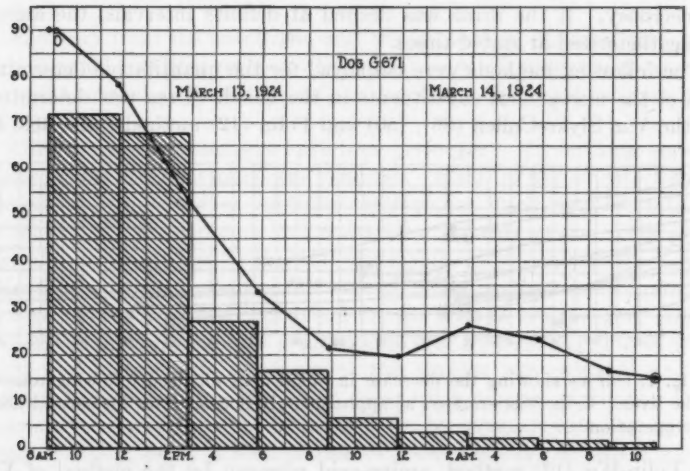


Fig. 2. Curve showing the decrease in blood urea nitrogen and the decrease in urine urea nitrogen following removal of the liver. The line *O* represents the blood urea nitrogen in milligrams for each liter of blood. The rectangles represent the milligrams of urea nitrogen excreted during each hour of the intervals when urine was collected. (See exper. 919.)

that the glucose is essential in restoring or maintaining the animal in normal condition.

Following hepatectomy there was an increased amount of amino-acids in the blood and urine, and even if amino-acids were given in large amounts over a period of several hours the urea in the blood and urine continued to decrease. The details of the effect of hepatectomy on the amino-acid content of the blood and urine will be discussed in a subsequent paper.

Urea estimations on the muscles, peritoneal fluid, gastric contents and so forth, at various times following hepatectomy, showed that the

urea content of these substances did not differ materially from that of the blood at the time the specimens were taken, and that a decrease in urea content of the tissues had occurred with the decrease in the urea content of the blood.

The second series of experiments comprises a group of twelve dogs in which the liver and kidneys were removed at the same time, and three dogs in which the entire contents of the peritoneal cavity were removed at the same time. These animals lived for from five to twelve hours after operation. The results in this series were similar to those in the previous series in which anuria followed hepatectomy. The amount of urea in the blood remained constant during the entire course of the experiments.

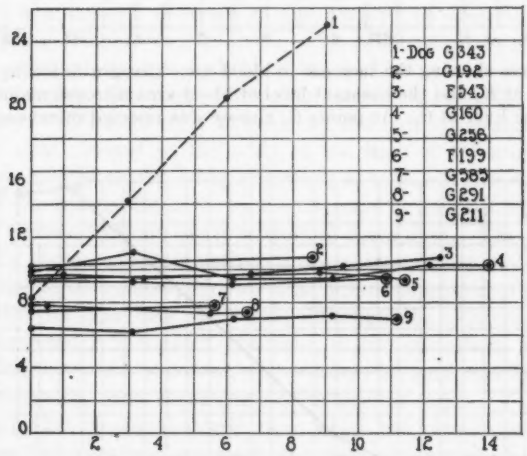


Fig. 3. Curves showing the constant level of blood urea nitrogen following simultaneous removal of liver and kidneys. Curve *1* represents a similarly treated animal in which the kidneys only were removed.

Tissue analysis revealed that the amount of urea in the muscles remained similar to that in the blood. The amino-acids of the blood increased markedly, but in no case was there any positive increase in the urea content of the blood.

In two animals, following removal of the kidneys there was the usual rise in the urea of the blood. When the liver was removed later, there was no further increase in the urea content of the blood, the amount of urea remaining unchanged from the time of hepatectomy until death.

Discussion. It should be emphasized that the results of these experiments are uncomplicated by the factors which have influenced most of the studies involving exclusion of the liver. The liver was removed rapidly

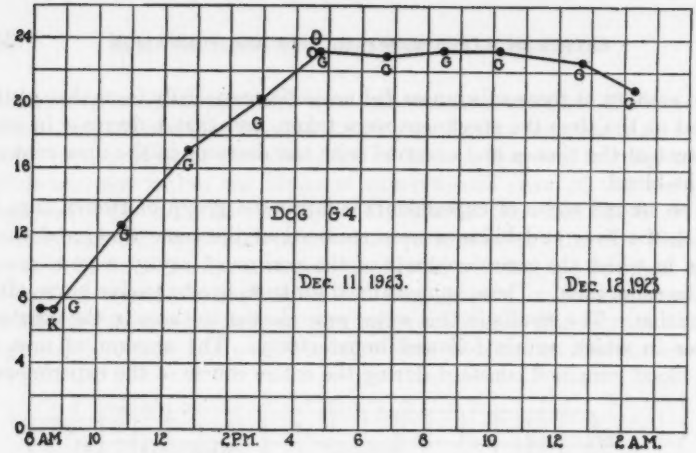


Fig. 4. Curve showing the increase in blood urea nitrogen following removal of both kidneys at *K*, and the constant level of blood urea nitrogen maintained after removal of the liver at *O*. At points *G*, glucose was injected intravenously. (See exper. 400.)

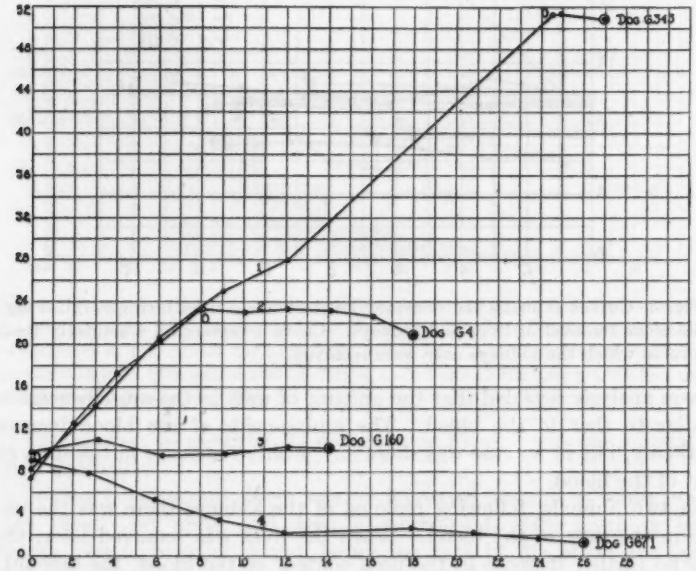


Fig. 5. Curves showing the effect of removal of the liver at *O*, on the blood urea nitrogen at different levels. Curve *1* shows the effect of removal of the liver at *O*, twenty-four hours after removal of both kidneys; curve *2*, the effect of removal of the liver eight hours after removal of both kidneys; curve *3*, the effect of simultaneous removal of the liver and both kidneys; and curve *4*, the effect of removal of the liver, urinary secretion being maintained.

with very little loss of blood, and the other tissues were not affected noticeably, either grossly or microscopically. Usually, within an hour after the beginning of the ether anesthesia the liver had been removed, the anesthetic discontinued, and the dog appeared normal. In many cases the dog continued to appear normal for as long as twenty-four hours. We consider a dog normal when it walks around, responds to call, wags its tail, and exhibits the usual interest in other dogs and in its surroundings. In brief the animal appears similar to one which has recovered from ether anesthesia and any surgical procedure.

The very striking decrease in the urea content of the blood and tissues, with the equally marked decrease in the urea excretion, proves conclusively that there is a marked decrease in the amount of urea formed in the body following removal of the liver. The progressive decrease in urea to such a minimal amount in both blood and urine is so marked as to indicate strongly that urea formation ceases immediately following hepatectomy. Although it is difficult to estimate accurately the total amount of urea present in the body at the time of operation, the total amount excreted from the body afterward is within a few milligrams of that which can be accounted for by the decrease in the urea content of the blood and tissues, without the formation of more urea. The excretion of ammonia by these animals is entirely inadequate to account for the diminution in the urea excreted, for the total ammonia excretion is markedly lowered with the low urea excretion, although the relative amount of ammonia excreted is increased. Further, in the animals in which both liver and kidneys were removed, the increase of ammonia in the blood was inconsiderable, and there was no increase in urea.

In animals that remained anuric, or in which the kidneys were removed at the time the liver was removed, the urea content of the blood and tissues remained constant until the conclusion of the experiment, as long as twelve hours later. All control animals, in which bilateral nephrectomy was performed, exhibited the usual marked rise in the urea content of the blood and tissues, but if the liver was subsequently removed from these animals no further rise could be detected, and the urea remained constant at this high level.

It may be suggested that much of the available substance for the formation of urea is mechanically removed with removal of the liver, since this organ has such marked power of absorption of amino-acids, and that removal of the liver thus reduces the animal to a low level of nitrogen metabolism, best comparable to that of an animal after several days' fasting. However, considerable amounts of amino-acids are present in the hepatectomized animal; there is a marked increase in the amino-acid nitrogen in the blood and urine of these animals, and injection of large amounts of amino-acids does not cause any change in urea. It

should also be remembered that the blood urea increased considerably during our experiments in dogs following bilateral nephrectomy alone, even after the nitrogen metabolism had been reduced to a minimum following fasting.

All of our evidence can be interpreted in no other manner than that no measurable amount of urea is formed following removal of the liver, and that the formation of urea in the body is absolutely dependent on the presence of the liver.

SUMMARY

Studies on the effect of total removal of the liver on the formation of urea have been made in more than ninety dogs, in which the results have not been complicated by loss of large amounts of blood or anesthesia while under observation. In many instances the animal appeared normal, walking around as long as twenty-four hours after operation.

In every case in which urine was secreted there was a marked decrease in the amount of urea in the blood and tissues, and also a marked progressive decrease of urea in the urine. If the animals were anuric or both kidneys were removed, the level of urea in the blood and tissues remained constantly the same as at the time of removal of the liver. This was true whether the urea content of the blood was high, as in the cases of removal of the liver some hours after nephrectomy, or the urea content was normal, as in the cases of removal of the liver and kidneys at the same operation, or the urea content was low, as in dogs that became anuric some time after the liver was removed when the urea content of the body had been depleted by excretion of the urea in the urine. Although the amino-acid content of the blood increases considerably following hepatectomy, no measurable amount of urea is formed in the body, even over a period of thirty-four and one-half hours after removal of the liver.

The production of urea in the body of the dog is entirely dependent on the presence of the liver, since urea formation ceases completely as soon as the liver is removed.

PROTOCOLS. *Experiment 520.* This is one of our earlier experiments which demonstrates the decrease in blood urea in a hepatectomized animal while the urinary outflow was maintained, and a constant low level of blood urea after the development of anuria.

Dog D850 was a male, adult mongrel shepherd, in good condition, and weighing 14.3 kgm. July 27, 1920, a reverse Eck fistula was performed. August 19, the animal weighed 14.7 kgm. The portal vein was ligated. September 27 the animal was still in excellent condition, except that he had lost weight. From the time of ligation of the portal vein until the liver was removed, he was fed on milk, syrup and bread.

September 28, at 8:30 a.m., the first blood specimen was obtained and the urine was examined. The liver was removed on this date. Anesthesia was started at

8:55 a.m., first incision made at 9:00 a.m., and the liver was out at 9:15 a.m. The animal was off the table at 9:30 a.m., and quite well recovered from the operation at 10:00 a.m. At 11:00 a.m. he was given glucose by rectum, which he did not retain very well.

At 1:00 p.m. the animal appeared to be in good condition. More glucose was given by rectum, but was only partially retained. At 2:30 p.m. the animal showed marked symptoms of muscular weakness; more glucose was given by rectum, but was not retained. At 3:30 p.m. the animal was perfectly flaccid except for muscular twitchings. Twenty cubic centimeters of 20 per cent glucose were injected intravenously. The animal recovered instantly, walked around, and appeared in good condition. At 4:20 p.m. the animal had again become weak, and the second blood specimen was taken. He was given 110 cc. of 20 per cent glucose. Recovery was prompt, and he walked around. At this time a weak solution of glucose was set before him, which he drank. At 5:00 p.m., 100 cc. of glucose were given by rectum, and were expelled a short time later, but the animal continued to drink glucose when given in a dilute form with water. At 7:00 p.m., 45 cc. of urine, containing a considerable amount of bile and also some sugar, were voided. At 10:30 p.m., 110 cc. of urine were voided. This also contained a large amount of bile and some sugar. The animal appeared to be in excellent condition, and although it vomited up a small amount of the glucose solution which it drank, a goodly share

TABLE 1

SPECIMEN NUMBER	UREA NITROGEN FOR EACH 100 CC.
	mgm.
1	9
2	7
3	4
4	3

was absorbed. Throughout the night following operation, he was in good condition. Gradually he ceased to drink the solution voluntarily, so small amounts of 40 to 50 cc. mixed with milk were placed in the mouth, and he swallowed them. His condition remained good.

At 4:30 a.m. the third blood specimen was obtained, and a few cubic centimeters of urine were voided. Throughout the day following removal of the liver, the animal was given glucose by mouth, by rectum and intravenously. He went into coma about 10:00 a.m., at 7:15 p.m. developed convulsions, and died at 7:45 p.m.

Necropsy (494) was performed at once. The site of operation was in good condition, and nothing of interest was noted. Practically no urine was found in the bladder, showing that the animal had been anuric for about sixteen hours.

Experiment 919. This experiment demonstrates the decrease in the blood urea and the decrease in urea excretion. The amount of urea excreted is approximately accounted for by the decrease in the urea in the blood and tissues, so that only minimal amounts, if any, of urea could have been formed in the twenty-six hours following hepatectomy.

Dog G671 was a female, adult mongrel, in good condition, and weighing 15.9 kgm. November 14, 1923, a reverse Eck fistula was performed. January 15, 1924, the animal weighed 15.8 kgm., and had remained in good condition since the operation. The abdomen was opened and the portal vein ligated. The intestines remained a fair color following the ligation, but the animal did not seem to have a

good chance of surviving. Accordingly, the spleen was also removed in order to cut down the amount of blood passing into the portal vein. March 13, 1924, the animal was still in good condition. On this date, the following experiment was performed:

At 8:50 a.m. the first blood specimen was taken. The bladder was empty. The animal was catheterized at 9:00 a.m., the incision made at 9:05 a.m., and the liver removed at 9:15 a.m. Ether was withdrawn at 9:30 a.m., the last stitch was taken at 9:32 a.m. The animal weighed 13.2 kgm. following operation; the liver weighed 339 grams. At 9:30 a.m., 27 cc. of 10 per cent glucose in 0.8 per cent sodium chlorid were injected intravenously. At 10:15 a.m. another injection was given. The animal was in good condition, but rather quiet. At 11:15 a.m. 27 cc. of 10 per cent glucose were injected intravenously. At 11:50 a.m. the second blood specimen was taken. The animal was catheterized, and 34 cc. of urine obtained. Twenty-seven cubic centimeters of 10 per cent glucose were injected intravenously.

At 2:00 p.m., 27 cc. of 10 per cent glucose were injected intravenously. At 2:50 p.m. the third specimen was taken, and the animalwas catheterized, 60 cc. of urine being obtained. Twenty-seven cubic centimeters of 10 per cent glucose were injected intravenously. At 4:15 p.m., 27 cc. of 10 per cent glucose were injected intravenously. At 5:20 p.m. the injection was repeated. At 5:50 p.m. the third specimen of urine was taken, 38 cc. Also the fourth specimen of blood was taken, and 27 cc. of 10 per cent glucose were injected. The animal was in good condition. At 7:10 p.m., 27 cc. of 10 per cent glucose were injected. At 8:00 p.m. the injection was repeated. The animal was in good condition. At 8:50 p.m. the fifth blood specimen was taken, and 36 cc. of urine were catheterized. The injection of 27 cc. of glucose was repeated. The animal was in good condition. At 9:45 p.m., 27 cc. of 10 per cent glucose were injected. The animal felt warm. The rectal temperature was 39.9°. There was a definite yellow tinge to the scleras. A slight amount of fluid was vomited. At 10:45 p.m., 27 cc. of 10 per cent glucose were injected. Some of this infiltrated. The portal vein was exposed, and 20 cc. more glucose solution were injected at 11:00 p.m. The rectal temperature was 39.7°. At 11:50 p.m. the fifth specimen of catheterized urine was obtained, 21 cc. The sixth blood specimen was taken, and 40 cc. of 10 per cent glucose were injected.

At 12:40 a.m., 30 cc. of 10 per cent glucose were injected. The animal was in good condition. At 1:55 a.m., 40 cc. of 10 per cent glucose were injected. The animal was in good condition, but had retched considerably during the last hour. At 2:50 a.m., the animal was in fair condition, but was retching considerably. Urine was catheterized, and the seventh blood specimen taken. Forty cubic centimeters of 10 per cent glucose solution were injected. At 4:00 a.m. the animal was restless and retching more. Another injection of 40 cc. of 10 per cent glucose solution was given. At 5:20 a.m. the animal was still restless and retching, but was otherwise in very good condition. He stood up in his cage, wagged his tail, and so forth. Another injection of 40 cc. of 10 per cent glucose solution was given. At 5:50 a.m. the animal was still restless, but in good condition. Urine was catheterized, and the eighth blood specimen (14.5 cc.) was taken. An injection of 30 cc. of 10 per cent glucose solution was given. At 6:50 a.m. the animal showed the first signs of the second stage, breathing hard with his nose against the cage. The 30 cc. injection was repeated. The rectal temperature was 40.4°. At 7:45 a.m., the animal seemed to be going into coma, but could still walk around and wag its tail. An injection of 40 cc. of 10 per cent glucose solution was given. At 8:50 a.m. the ninth blood specimen was taken, and 5 grams of glucose, 1 gram of sodium sulphate and 0.5 gram of sodium bicarbonate in 100 cc. of normal salt solution were

injected. Eighteen cubic centimeters of urine were catheterized. The rectal temperature was 41.2°. The animal was definitely comatose; the pulse and respiration were regular, but somewhat rapid. At 9:40 a.m., 150 cc. of 10 per cent glucose solution were injected. Urine was catheterized (6 cc.). At 10:40 a.m., 20 cc. of 50 per cent glucose solution were injected. The animal died at 10:50 a.m. The urine was taken from the bladder (17 cc.) and added to the 6 cc. catheterized at 9:40 a.m. The tenth blood specimen was taken from the heart.

Necropsy (189) was performed at once. The scleras and mucous membranes were definitely yellow, and the fat was very yellow. About 200 cc. of blood-stained fluid were found in the peritoneal cavity. About 50 cc. of stomach contents were added to the vomitus which was collected during the experiment. The intestines contained not more than 20 cc. of fluid in all. The liver and spleen had been completely removed. The other organs showed no gross changes, and specimens were saved for section. A muscle specimen was taken from the vastus medialis. The vomitus and stomach contents amounted to 415 cc.

Experiment 563. This experiment demonstrates the constant level of urea in the blood following simultaneous removal of the liver and both kidneys.

Dog F457 was a male, adult shepherd, in good condition, weighing 18.1 kgm. May 23, 1922 a reverse Eck fistula was performed. October 9, the dog weighed 22.7 kgm. A median incision was made and the portal vein ligated. Following the ligation, the intestines remained a good color. October 25, the animal was still in good condition. On this date the following experiment was performed:

At 8:20 a.m. the first blood specimen was taken. At 8:30 a.m., the animal was etherized; the incision was made at 8:40 a.m., and the liver removed at 8:50 a.m. Ether was withdrawn at 9:15 a.m., and the last stitch was taken at this time. The kidneys were also removed. The animal weighed 22.2 kgm. postoperatively; the liver weighed 390 grams. At 9:15 a.m., immediately after the operation, the second blood specimen was taken. At this time 0.5 gram of glucose for each kilogram of body weight was injected through a jejunostomy tube. At 10:15 a.m. the injection of glucose was repeated. At 11:15 a.m. the injection was repeated.

At 12:15 p.m., the third blood specimen was taken. An injection of 0.5 gram of glucose for each kilogram of body weight was given intravenously. At 1:45 p.m., 0.5 gram of glucose for each kilogram of body weight was injected through a jejunostomy tube. At 3:15 p.m. the fourth blood specimen was taken, and an injection of 0.5 gram of glucose for each kilogram of body weight was made through a jejunostomy tube, and another 0.5 gram given intravenously. At 4:15 p.m. the injection of glucose through the tube was repeated. At 5:15 p.m., 0.5 gram of glucose for each kilogram of body weight was injected through the tube intravenously. At 6:15 p.m., the fifth blood specimen was taken. At 7:00 p.m. 0.5 gram of glucose for each kilogram of body weight was injected through the tube. At 8:15 p.m., the injection through the tube was repeated. At 9:15 p.m. the sixth specimen was taken. The animal died immediately after the completion of the injection intravenously of 0.5 gram of glucose for each kilogram of body weight.

Necropsy (728) was performed immediately after death. There was no hemorrhage. Nothing was found which would in any way invalidate the results of the experiment.

Experiment 400. This experiment demonstrates the increase in blood urea following removal of both kidneys, and the constant level of blood urea subsequent to removal of the liver.

Dog G4 was a male, adult mongrel, in good condition, and weighing 19.7 kgm. May 3, 1923 a reverse Eck fistula was performed through a median line incision. August 13, the animal was still in excellent condition, and it weighed 20.6 kgm.

TABLE 2

Blood analyses for dog G671, experiment 919

SPECIMEN NUMBER	TIME	UREA NITROGEN	AMINO-ACID NITROGEN	URIC ACID	NON-PROTEIN NITROGEN
		mgm.	*mgm.*	*mgm.*	*mgm.*
1	8:50 a.m.	9.0	8.2	0.50	29.5
2	11:50 a.m.	7.8			
3	2:50 p.m.	5.3			
4	5:50 p.m.	3.35	10.4	1.25	25.8
5	8:50 p.m.	2.15			
6	11:50 p.m.	1.98			
7	2:50 a.m.	2.67			
8	5:50 a.m.	2.32			
9	8:50 a.m.	1.68	11.6	3.55	30.5
10	10:50 a.m.	1.52	11.3	3.80	30.8

TABLE 3

Urine, total amounts for dog G671, experiment 919

SPECIMEN NUMBER	TIME	VOLUME	AMMONIA AND UREA NITROGEN	AMMONIA NITROGEN	UREA NITROGEN	AMINO-ACID NITROGEN	URIC ACID	BILE PIGMENT
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
1	11:50 a.m.	34	234	18.6	215.4	20.9	204	0
2	2:50 p.m.	60	259	55.2	203.8	47.6	360	++
3	5:50 p.m.	38	129	47.5	81.5	41.8	239	++
4	8:50 p.m.	36	62.6	12.5	50.1	25.1	223	++
5	11:50 p.m.	21	29.0	10.7	18.3	19.9	126	++
6	2:50 a.m.	17	17.85	8.5	9.35	15.7	105	++
7	5:50 a.m.	14.5	13.7	6.6	7.1	14.8	87	++
8	8:50 a.m.	18	12.96	7.5	5.46	15.8	106	++
9	10:50 a.m.	23	10.46	7.8	2.66	15.6	85	++
Total in 26 hours.....		261.5	768.6	174.9	593.7	217.2	1535	++

Urea content

SPECIMEN NUMBER	VOLUME	CONCENTRATION FOR EACH 100 CC. URINE					UREA AND AMMONIA NITROGEN	
		Ammonia and urea nitrogen	Ammonia nitrogen	Urea nitrogen	Amino-acid nitrogen	Uric acid	Urea nitrogen	Ammonia nitrogen
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
1	34	685	45.0	640.0	61.6	600	93.4	6.6
2	60	432	92.0	340.0	79.3	600	78.5	21.5
3	38	340	125.0	215.0	110.0	630	63.3	36.7
4	36	174	34.8	139.2	72.6	620	80.0	20.0
5	21	138	51.0	87.0	95.0	600	71.0	29.0
6	17	105	50.0	55.0	92.5	620	52.4	47.6
7	14.5	95	45.8	49.2	102.0	600	52.0	48.0
8	18	72	41.5	30.5	88.0	590	42.4	57.6
9	23	45.5	33.5	12.5	77.0	370	26.4	73.6

The abdomen was opened, and it was found that there was practically no collateral circulation established. The portal vein was large. Following its ligation, the intestines became blue. The ligature was accordingly removed, and the vein was about half occluded by a blood vessel suture. The intestines remained pink following the partial ligation. October 12, the animal was still in good condition. He weighed 21.3 kgm. The ligation of the portal vein was completed. December

TABLE 4

Dog G671, experiment 919

Muscle A—2.350 grams = 1.66 mgm. urea plus ammonia nitrogen for each 100 grams.
Muscle B—2.150 grams = 1.70 mgm. urea plus ammonia nitrogen for each 100 grams.

Peritoneal fluid— 5 cc. = 1.65 mgm. urea plus ammonia nitrogen for each 100 cc.
Peritoneal fluid—200 cc. = 3.30 mgm. urea plus ammonia nitrogen total.

Vomitus and gastric contents— 5 cc. = 5.6 mgm. urea and ammonia nitrogen for each 100 cc.
Vomitus and gastric contents—415 cc. = 28.24 mgm. urea and ammonia nitrogen total.

Total urea excreted	593.7 mgm.
Total urea vomitus	23.24 mgm.
Total urea peritoneal fluid	3.3 mgm.
	620.24 mgm.
Ammonia nitrogen excreted	174.9 mgm.
	795.14 mgm.

TABLE 5

Blood analyses for dog F457, experiment 563

SPECIMEN NUMBER	TIME	SUGAR	TOTAL NON-PROTEIN NITROGEN	UREA NITROGEN	MI NO-ACID NITROGEN	AMMONIA NITROGEN	URIC ACID	CREATI-NIN	TOTAL CREATI-NIN
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8:20 a.m.	0.084	22.7	9.5	5.43	0.06	Trace	1.61	3.53
2	9:45 a.m.	0.065	22.9	9.7	4.56		1.14	1.64	3.94
3	12:15 p.m.	0.083	24.8	9.4	6.90	0.05	2.12	2.16	
4	3:15 p.m.	0.056	25.9	9.8	7.50	0.04	2.76	2.75	4.45
5	6:15 p.m.	0.088	26.9	10.2	8.91	0.17	3.96	3.33	4.79
6	9:15 p.m.	0.116	27.7	10.4	9.45	0.18	5.18	3.98	4.81

11, the animal was still in good condition, and weighed 16.6 kgm. The following experiment was performed:

At 8:15 a.m. the first blood specimen was taken. The animal was then etherized, and both kidneys were removed at 8:45 a.m. They weighed 150 grams. At 9:00 a.m. 0.5 gram of 25 per cent glucose solution was injected. At 10:45 a.m. the second blood specimen was taken; an injection of 0.5 gram of 50 per cent glucose for each kilogram of body weight was made.

At 12:45 p.m. the third blood specimen was taken. An injection of 0.5 gram of 50 per cent glucose for each kilogram of body weight was made. At 3:00 p.m. the fourth blood specimen was taken, and the injection repeated. At 4:20 p.m. the animal was again etherized. The incision was made at 4:30 p.m., and the liver was removed at 4:40 p.m. Ether was withdrawn at 4:55 p.m., and the last stitch was taken at this time. The liver weighed 411 grams, and the animal weighed 15.9 kgm. postoperatively. At 4:45 p.m. the fifth blood specimen was taken. An injection of 0.5 gram of 50 per cent glucose for each kilogram of body weight was made. At 6:45 p.m. the sixth blood specimen was taken, and the injection repeated. The animal showed marked twitchings at this time, but made a fair recovery after the injection. At 8:30 p.m. the seventh blood specimen was taken. The animal was unable to stand. An injection of 0.5 gram of 25 per cent glucose for each kilogram of body weight was made, also 0.25 gram of 50 per cent solution in 42 cc. of sodium chlorid. At 10:10 p.m. the eighth specimen was taken. The animal was unable to stand. An injection of 0.5 gram of 50 per cent glucose diluted in equal parts of sodium chlorid solution, for each kilogram of body weight was made. At 11:20 p.m., 0.5 gram of 50 per cent glucose in equal parts of sodium chlorid was injected.

TABLE 6

Blood analyses for dog G4, experiment 400

SPECIMEN NUMBER	TIME	SUGAR	NON-PROTEIN NITROGEN	UREA NITROGEN	AMINO-ACID NITROGEN	URIC ACID	CREATININ
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8:15 a.m.	0.098	29.2	7.35	7.72	0.89	1.16
2	10:45 a.m.			12.50			
3	12:45 p.m.	0.073	39.1	17.2	6.78	1.49	2.12
4	3:00 p.m.			20.2			
5	4:45 p.m.	0.067	44.5	23.3	7.10	1.24	3.75
6	6:45 p.m.			22.9			
7	8:30 p.m.	0.040	47.8	23.3	8.32	3.96	5.45
8	10:10 p.m.			23.0			
9	12:45 a.m.			22.5			
10	2:15 a.m.	0.030	49.8	21.0	11.10	8.90	8.63

At 12:45 a.m. the ninth blood specimen was taken; the animal vomited approximately 300 cc. of fluid. The injection of glucose was repeated. At 2:15 a.m. the animal died. The tenth blood specimen was taken immediately after death.

Necropsy (678) was performed immediately after death. The peritoneal cavity contained very little free fluid. Nothing was found which would in any way invalidate the results of the experiment.

BIBLIOGRAPHY

(1) BAYLISS: Principles of general physiology. London, 2 ed., 1918, p. 265.
(2) BERNARD AND BARRESWIL: Arch. gen. de med., 1847, 4s., xiii, lxxiii, 449.
(3) BIEDL AND WINTERBERG: Pflüger's Arch., 1902, lxxxviii, 140.
(4) CATHCART: Biochem. Journ., 1916, x, 197.
(5) CYON: Centralbl. f. d. med. Wissensch., 1870, 580.
(6) DOYON AND DUFOURT: Arch. de physiol. norm. et path., 1898, 5s., x, 522.

(7) Embden, Salomon and Schmidt: Beitr. z. chem. Phys. u. Path. 1906, viii, 129.
(8) Felix and Tomita: Zeitschr. f. physiol. Chem., 1923, cxxviii, 40.
(9) Fiske and Karsner: Journ. Biol. Chem., 1913–14, xvi, 399.
(10) Fiske and Karsner: Journ. Biol. Chem., 1914, xviii, 381.
(11) Fiske and Sumner: Journ. Biol. Chem., 1914, xviii, 285.
(12) Folin: Laboratory manual of biological chemistry, with supplement. New York, Appleton, 3rd ed., 1922, 300 pp.
(13) Folin: Physiol. Reviews, 1922, ii, 460.
(14) Folin and Berglund: Journ. Biol. Chem., 1922, li, 395.
(15) Folin and Denis: Journ. Biol. Chem., 1912, xi, 87; Journ. Biol. Chem., 1912, xii, 141.
(16) Folin and Denis: Journ. Biol. Chem., 1912, xi, 161.
(17) Fosse and Rouchelman: Compt. rend. Acad. d. sci., 1921, clxxii, 771.
(18) Frerichs: Pathologisch-anatomischer Atlas zur Klinik der Leberkrankheiten, 1861, l, 240.
(19) Frey: Zeitschr. f. klin. Med., 1911, lxxii, 383.
(20) Fürth: Probleme der physiologischen und pathologischen Chemie. Leipzig, 1913, ii, 100.
(21) Gilbert, Chambrol and Bénard: Paris méd., 1920, xxxv, 385.
(22) Gréhant: Dosage de l'urée à l'aide du réactif de Millon et de la pompe à mercure. Paris, 1872, 4 pp.
(23) Gréhand and Quinquaud: Comp. rend. Soc. de biol., 1881, 162.
(24) Gscheidlen: Studien über den Ursprung des Harnstoffs im Thierkörper. Leipzig, 1871, 44 pp.
(25) Hahn, Massen, Nencki and Pawlow: Arch. de. sc. biol., 1892, i.
(26) Hammett: Journ. Biol. Chem., 1919, xxxviii, 105.
(27) Hawk: Practical physiological chemistry. Philadelphia, 8 ed., 1923, p. 398.
(28) Hench and Aldrich: Journ. Amer. Med. Assoc., 1922, lxxix, 1409.
(29) Herter: Johns Hopkins Hosp. Rept., 1900, ix, 69.
(30) Herter and Wakeman: Journ. Exper. Med., 1899, iv, 117.
(31) Heynsius: Arch. f. Holländischer Beiträge zur Natur und Heilkunde, 1859, i, 303.
(32) Hoagland and Mansfield: Journ. Biol. Chem., 1917, xxxi, 487.
(33) Hoppe-Seyler and Pekelharing: Zeitschr. f. physiol. Chem., 1881, v.
(34) Horodynski, Salaskin and Zaleski: Zeitschr. f. physiol. Chem., 1902, xxxv, 246.
(35) Howell: Textbook of physiology. Philadelphia, 8 ed., 1922, p. 825.
(36) Jansen: Journ. Biol. Chem., 1915, xxi, 557.
(37) Jansen: Physiol. Abstr., 1916–1917, i, 23.
(38) Kaufmann: Arch. de physiol. norm. et path., 1894, 5 s., vi, 531.
(39) Knieriem: Beiträge zur Kenntniss der Bildung des Harnstoffs im thierischen Organismus. Dorpat, Mattiesen, 1874, 32 pp.
(40) Kossel and Dakin: Zeitschr. f. physiol. Chem., 1904, xli, 321.
(41) Kossel and Dakin: Zeitschr. f. physiol. Chem., 1904, xlii, 181.
(42) Levene and Meyer: Journ. Biol. Chem., 1913–1914, xvi, 555.
(43) Lieblein: Arch. f. exper. Path. u. Pharm., 1893-1894, xxxiii, 318.
(44) Löffler: Physiol. Abstr., 1916–1917, i, 477.
(45) Macleod: Physiology and biochemistry in modern medicine. St. Louis, 2nd ed., 1919, p. 617.
(46) Mann: Amer. Journ. Med. Sci., 1921, clxi, 37; This Journal, 1921, lv, 285.
(47) Mann and Magath: Trans. Sect. Path. and Physiol., Amer. Med. Assoc., 1921, 29.

(48) MARCHAND: Repertoire de chim. de Gaultier de Claubry, 1838, iii.
(49) MARCHAND: Journ. L'Experience, 1839, ii, 43.
(50) MARSHALL: Journ. Biol. Chem., 1913, xiv, 283; 1913, xv, 487, 495; 1914, xvii, 351.
(51) MARSHALL AND DAVIS: Journ. Biol. Chem., 1914, xviii, 53.
(52) MATHEWS: Physiological chemistry. New York, 3rd ed., 1920, 1154 pp.
(53) MATTHEWS AND NELSON: Journ. Biol. Chem., 1914, xix, 229.
(54) MEISSNER: Zeitschr. f. rat. Med., xxvi, 225; 1868, xxxi, 234, 283.
(55) MEISSNER: Zeitschr. f. rat. Med., 1868, xxi, 234.
(56) MINKOWSKI: Arch. f. exper. Path. u. Pharm., 1886, xxi, 41.
(57) MOLESCHOTT: Arch. f. physiol. Heilkunde, 1852, xi, 492.
(58) MUNK: Pflüger's Arch., 1875, xi, 100.
(59) NASH AND BENEDICT: Journ. Biol. Chem., 1921, xlviii, 463.
(60) NEBELTHAU: Zeitschr. f. Biol., 1888–1889, n. f. vii, 123.
(61) PEKELHARING: Arch. f. Physiol., 1875, xi, 602.
(62) PERRONCITO: Riforma med., 1920, xxxvi, 830.
(63) PICARD: Compt. rend. Soc. de biol., lxxxiii, 1876.
(64) PICARD: Journ. de l'anat. et physiol., 1881, xvii, 530.
(65) PICARD: Thèse de Strasbourg, 1856, no. 375, xxii, 96 pp.
(66) POISEUILLE AND GOBLEY: Compt. rend., 1859, xlix, 164.
(67) POULSSON: Arch. f. exper. Path. u. Pharm., 1892, xxix, 244.
(68) PREVOST AND DUMAS: Ann. de chim. et de phys., 1823, xxiii, 90.
(69) RICH: Bull. Johns Hopkins Hosp., 1923, xxxiv, 321.
(70) ROUELLE: Journ. de med., 1773 (Nov.).
(71) SALASKIN: Zeitschr. f. physiol. Chem., 1898, xxv, 128.
(72) SALASKIN: Zeitschr. f. physiol. Chem., 1898, xxv, 449.
(73) SCHMIEDEBERG: Arch. f. exper. Path. u. Pharm., 1878, viii, 1.
(74) SCHÖNDORFF: Pflüger's Arch., 1899, lxxiv, 307.
(75) SCHRÖDER: Arch. f. exper. Path. u. Pharm., 1882, xv, 364.
(76) SCHRÖDER: Arch. f. exper. Path. u. Pharm., 1885, xix, 373.
(77) SCHRÖDER: Zeitschr. f. physiol. Chem., 1890, xiv, 576.
(78) SLOSSE: Arch. f. Physiol. (Dubois Raymond), 1890, 482.
(79) STADIE AND VAN SLYKE: Arch. Int. Med., 1920, xxv, 693.
(80) STÄDELER AND FRERICHS: Journ. f. prakt. Chem., 1858, lxxiii, 48.
(81) STOKVIS: Arch. f. Holländischer Beitr. z. Natur. und Heilkunde, 1860, ii, 260.
(82) TAYLOR AND LEWIS: Journ. Biol. Chem., 1915, xxii, 77.
(83) TIEDEMANN AND GMELIN: Zeitschr. f. Physiol., xiv.
(84) VAN SLYKE: Harvey Lecture, 1915–1916, xi, 146.
(85) VAN SLYKE: Journ. Biol. Chem., 1912, xii, 275; 1913, xvi, 121, 125.
(86) VAN SLYKE AND CULLEN: Journ. Amer. Med. Assoc., 1914, lxii, 1558.
(87) VAN SLYKE AND MEYER: Journ. Biol. Chem., 1913–1914, xvi, 213.
(88) WELLS: Chemical pathology. Philadelphia, 4 ed., 1920, p. 533.
(89) WINTERBERG AND MÜNZER: Arch. f. exper. Path. u. Pharm., 1894, xxxiii, 164.

STUDIES ON THE PHYSIOLOGY OF THE LIVER

IX. The Formation of Bile Pigment after Total Removal of the Liver

FRANK C. MANN, JESSE L. BOLLMAN and THOMAS B. MAGATH

From the Division of Experimental Surgery and Pathology, Mayo Foundation, Rochester, Minnesota

Received for publication April 22, 1924

One of the first and most striking observations we made on our totally hepatectomized animals was the development in the urine, plasma and fat, of a pigment similar to that found in bile. A specimen of urine obtained from the hepatectomized animal shortly after operation had a tinge identical with the color when the urine contained bile. If the animal lived for more than six hours, a yellow tinge was also found in the plasma, and at necropsy the color of the fatty tissues was a dirty yellow. After we had found that the immediate cause of death in the hepatectomized animal was associated with a condition of hypoglycemia, and that glucose properly administered would maintain life for a much longer period, we were able to follow the development of this pigment to better advantage (21). By the employment of many tests in these early experiments we satisfied ourselves that this pigment was bilirubin, or at least so closely related that it could not be distinguished from it. Our results, in general, confirmed the accepted results at that time (38), and therefore, aside from making a brief statement of our findings (20), and conducting routine observations on the development of the pigment in all the hepatectomized animals, we devoted ourselves to other phases of the effect of total removal of the liver. The recent discussion in the literature (26) of the relation of the liver to the formation of bilirubin has made it desirable to place on record our observations on this phase of our studies on the effect of removal of the liver.

Previous work on the extrahepatic formation of bilirubin. Müller (25) removed the livers of frogs and studied the blood and tissues for the presence of bile pigment. He was unable to detect bile pigment in the blood or tissues of frogs that lived four days after hepatectomy. Kunde (13) removed the livers from 250 frogs that lived longer than four days, and found in their blood a green pigment which he extracted with alcohol, and which turned red on the addition of nitric acid. He thought that this pigment was a precursor of bilirubin, normally excreted by the liver.

Moleschott (24) kept his frogs alive as long as twenty-one days after the liver had been removed, and could not find pigment in their blood or tissues. He did show that Kunde's pigment was bilirubin which had been absorbed from the intestine and could easily be found after four days, but after eight days the cloaca was free from pigment. However, Minkowski and Stern (23) ligated the common duct in frogs, and for six weeks afterward could find no trace of bilirubin. Bile pigment was not found, by any test, in the blood and urine of these animals, and icterus was not observed except after the administration of hemoglobin, toluylendiamin or arsenated hydrogen.

Stern, using the pigeon, which secretes bilirubin in the urine in 1.5 hours, and has bile pigment in the blood five hours after ligation of the common duct, could not find a trace of icterus after ligation of all the blood to the liver for as long as twenty-four hours.

Minkowski and Naunyn (22) removed the livers from hens, ducks and geese, and could find only traces of bile pigment in their urine. They concluded that the slight amount of pigment in the blood was due to the absorption of the bile pigment present in the intestine. They also found that icterus following toluylendiamin was entirely prevented by the removal of the liver.

McNee (18) repeated the work of Minkowski and Naunyn on geese with similar results. He called attention to the difference in the structure of the liver in birds and mammals, and attributed the lack of formation of pigment to the removal of the Kupffer cells. In the cases in which small amounts of pigment were observed, he concluded that the Kupffer cells in the spleen and bone marrow were still active. He believed that a true hematogenous jaundice in birds without livers was impossible.

Vögel (37) used ducks and geese and could not obtain any icterus even after the administration of toluylendiamin in his hepatectomized animals.

Whipple and Hooper (38) in studying the jaundice that develops in dogs following injections of hemoglobin, or following chloroform poisoning, found that dogs with Eck's fistula developed icterus similar to that in normal dogs under the same conditions. The depletion of the blood supply to the liver did not prevent or delay the occurrence of icterus, although it was diminished. The authors concluded that the liver could not be the essential factor in the conversion of hemoglobin to bilirubin, and attempted to prove this conclusively in their next series of experiments (39). In dogs with Eck's fistula, positive reactions for bilirubin were obtained in the urine from one to one and one-half hours after ligation of the hepatic artery and the injection of hemoglobin. In two such experiments positive reactions were obtained on the blood three hours and forty minutes, and five hours and thirty minutes after injection of hemoglobin, and the fat of these animals gave positive reactions at the

same intervals. When the spleen was removed from an animal and the foregoing experiment performed, positive reactions for bile pigment were obtained on the urine in two hours and thirty minutes, and on the fat after five hours, but the blood remained negative for five hours, the duration of the experiment. Finally positive reactions for bilirubin in the blood and fat of dogs, with the head-thorax circulation only, were obtained in from two to five hours after the injection of hemoglobin. The description of the icterus appearing in these dogs, especially in the fat, is the typical description of the jaundice we observe in all of our dogs surviving hepatectomy more than six hours.

Mann and Magath (20 b), in a brief report, have called attention to the constant findings of icterus in dogs that survive total removal of the liver for more than six hours. The plasma of these animals became definitely yellow, and the subcutaneous, pericardial and mesenteric fat became a characteristic dirty yellow in contrast to the normal white fat of the dog. Recently Bickel (3), using the same method of removing the liver from dogs, observed the same development of jaundice.

Rich, in repeating Whipple and Hooper's work, removed the entire abdominal viscera (liver, spleen, stomach, intestines, pancreas and kidneys) and filled the space with warm towels. Of the eight experiments reported, seven animals survived for from one hour to two hours and ten minutes, while the eighth survived five hours and twenty minutes, following the injection of laked red cells. In none of these animals was bilirubin detected during or at the conclusion of the experiment, either in the blood or subcutaneous fat. Rich then repeated Whipple and Hooper's technic, and found that after injecting India ink in the external jugular vein, or into the aorta, the ink made its way into the liver through the vessels in the diaphragm which enter the liver along the wall of the inferior vena cava. His experiments demonstrated that the liver after the ligation, as practiced by Whipple and Hooper, may still have both arterial and venous circulation, although obviously but a small part of the blood can pass that way. Rich ligated the aorta, celiac axis, mesenteric arteries, portal vein and hepatic artery, and finally the vena cava above the liver. The injected ink no longer found its way to the liver, thus showing that all the blood supply to the liver was shut off. Under morphin, these animals survived the injections of hemoglobin as long as three hours and twenty minutes, and the formation of bilirubin could not be demonstrated. From these experiments he concluded that the liver was essential for the conversion of circulating hemoglobin to bile pigment.

McNee and Prusik (19) were likewise unable to confirm the results of Whipple and Hooper in the majority of their experiments. Hemoglobin was introduced in six "heart-lung preparations" of Starling, and no bilirubin was detected at the conclusion of the experiments, thirty minutes

to five hours and fifty minutes later. Seven negative "head-thorax circulation" experiments are reported which lasted from thirty-five minutes to two hours and forty-five minutes. Two head-thorax circulation experiments were positive for the formation of bilirubin; one animal developed one unit, by the Van den Bergh (35) method, of bilirubin in the circulating blood in one hour and ten minutes after the injection of horse hemoglobin; in the second, the reaction was faintly positive after hemoglobin from the laked blood of the same dog was allowed to circulate for two hours and eighteen minutes. The authors concluded that the site of origin of the bile pigment in hemolytic jaundice must be considered as undetermined.

Localized transformation of hemoglobin to bile pigment has been known since the observations of Virchow (36) on hematoidin, an isomer of bilirubin, and although the subsequent work reviewed here does not relate to the effect of exclusion or removal of the liver, it is given because it seems to be more or less indicative of the extrahepatic formation of bile pigment. Bilirubin has been found around the site of extravasated blood in all parts of the body by many observers. Froin, in examining 178 hemorrhagic fluids from the peritoneum, pleura and cerebro-spinal fluid, found bilirubin present in fifty-three cases. He concluded that the formation of bilirubin was local, due to the action of leucocytic ferments. Whipple and Hooper (40) were able to demonstrate the formation of considerable quantities of bilirubin from hemoglobin injected into the peritoneal or pleural cavity of dogs. Van den Bergh (34) has found the bilirubin content of old hematomas to be enormous, as in a case of traumatic subdural hematoma which contained bilirubin in 1:4,000 concentration, which actually corresponds to the concentration he reports in a case of bile from a fistula of the gallbladder.

Jones and Jones found bile pigment in the blood in the arm constricted with a tourniquet following an attack of paroxysmal hemoglobinuria. They concluded that the liver was not essential for the conversion of hemoglobin to bilirubin. Van den Bergh (34), Lepehne (14) and others have confirmed the assertion of Gilbert that normal human serum contains bilirubin and the demonstration of the normal presence of bilirubin in the blood normally, has led both Roger and Brulé to suggest that it appears in the blood like the urea on its way to the excreting organ, and that bilirubin is to the liver as urea is to the kidney.

Tarchanoff found that bilirubin was promptly excreted in the bile of dogs with fistulas that were injected with bilirubin. He also observed that the excretion of bilirubin increased following the injection of hemoglobin, and concluded that the bilirubin was formed in the blood, since he found it in the urine after hemoglobin injections, the same as following bilirubin injections.

Stadelmann found that injected bilirubin was excreted mostly in the bile within two to four hours after injection, but that the injection of hemoglobin did not alter the amount of bilirubin in the bile until from three to four hours after injection when the increase began to be noticed. Brugsch and his collaborators have made similar observations following the injection of hematoporphyrin and hematin, and even found urobilin to be converted to bilirubin or biliverdin. McMaster, Broun and Rous found that any condition leading to increased blood destruction gave rise to an increased excretion of bile pigment by the liver, but they found no quantitative relationship. Rosenthal and Meier found that the icterus following toluylendiamin phenylhydrazin or phosphorus in dogs, bore no relation to the amount of blood destruction.

The importance of the reticulo-endothelial system in the formation of bile pigment has been emphasized, especially by Aschoff and his school. They have shown that these cells are especially active in the destruction of hemoglobin, either from broken-down blood cells or free hemoglobin in the blood. Lepehne and McNee have considered that these cells are the sites of the formation of bilirubin, and that icterus arises from the increased activity of these cells, or to obstruction of the excreting cells in the liver. Schilling and Rosenthal and Melchior express doubts with regard to the activity of the reticulo-endothelial cells in the formation of bile pigments. The latter found that jaundice developed in pigeons following administration of arsenated hydrogen, even though the Kupffer cells had been previously poisoned with colloidal silver.

This brief review clearly indicates that there is, at present, no general agreement with regard to the site of formation of bilirubin, or the part the liver plays in the process. There are many indications pointing to an extrahepatic origin of bile pigment. It should be noted that Whipple and Hooper (38), and to a less extent McNee and Prusik, were the only investigators who obtained positive data on the formation of bilirubin after removal of the liver from the mammal.

METHODS. The liver was totally removed, employing the technic previously described (21). In this connection the following should be emphasized: the liver is totally removed; the final operation occupies only a short anesthetic period; there is usually very little blood lost, and only a relatively small amount of operative trauma; the circulation from the abdominal viscera and posterior extremities is maintained; the animal quickly recovers from the anesthesia and operation; the animal is maintained in a normal appearing condition for many hours after the total removal of the liver, by the proper administration of glucose. By "normal appearing," we mean that the animal can walk around, respond normally to call, and in appearance cannot be differentiated from any other dog which has recently undergone an operation.

The experiments can be grouped in four series. In the largest series, the liver was removed and the development of the pigment followed more or less routinely, qualitatively and some times quantitatively. Most of the experiments were performed primarily for the purpose of observing other effects of the total loss of the liver. In one series of experiments hemoglobin was injected intravenously after hepatectomy in an attempt to hasten the formation of pigment. In another series of experiments the spleen was removed at the same time, or previous to the removal of the liver. In a few experiments, a reverse Eck fistula was performed, and at a later date, all the abdominal viscera were removed. It should be noted that in this series of experiments the portal circulation through the liver was increased before removal of the liver as contrasted with the other series in which it was abolished.

The urine, plasma and extract of fatty tissue were tested, employing the more generally accepted tests for bilirubin. These included the Gmellin, Adler, Herzfeld, Rosenberg, Huppert-Cole and Van den Bergh (35). For quantitative work the Van den Bergh test was employed. The preparations of hemoglobin were made from the animal's own laked blood, or from the blood of other dogs.

Results. A specimen of urine obtained from a hepatectomized animal a few hours after operation was bile-tinged, and gave a positive reaction for bilirubin. In from three to six hours after removal of the liver the plasma becomes tinged with yellow. This color usually increases progressively until death, and the reaction for bilirubin is positive. A yellow tinge also develops in the sclera in the animals that live sixteen hours more after operation, and can also sometimes be seen in the mucous membranes. At necropsy all the fatty tissue is dirty yellow. The chloroform extract of this fatty tissue is yellow and gives a positive reaction for bilirubin. This yellow pigment, wherever found, was positive for bilirubin by all the tests that we have employed. The Van den Bergh (35) is first indirect and later may become biphasic (6). The removal of the spleen, either at the same time or previous to removal of the liver, in no way affects the development of the pigment. The pigment also develops when the entire abdominal viscera are removed. The injection of hemoglobin some hours after hepatectomy, when pigment is already present in the plasma, seems to produce a definite increase in its formation. The pigment will develop when no blood is present in any of the serous cavities although it seems to develop faster when some free blood is present in the abdominal cavity (figs. 1 to 3).

Our observations include more than a hundred hepatectomized dogs in which the pigment developed; it was found in all animals living longer than six hours. The time of appearance of the pigment after operation and the rate at which the pigment has developed has varied in the different

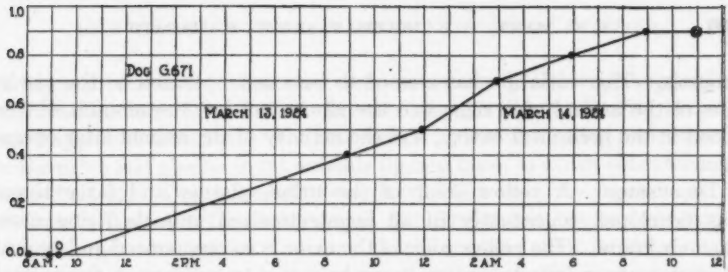


Fig. 1. Curve showing the accumulation of bilirubin in the blood following total removal of the liver, *O*. This animal also eliminated considerable amounts of bile pigment in the urine during the course of the experiment. (Exper. 919–23 and table 3.)

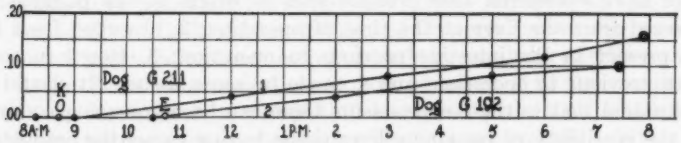


Fig. 2. Curve showing the accumulation of bilirubin in the blood following removal of the liver, *O*, and kidneys, *K* (curve *1*) and removal of all the intra-abdominal viscera, *E* (curve *2*).

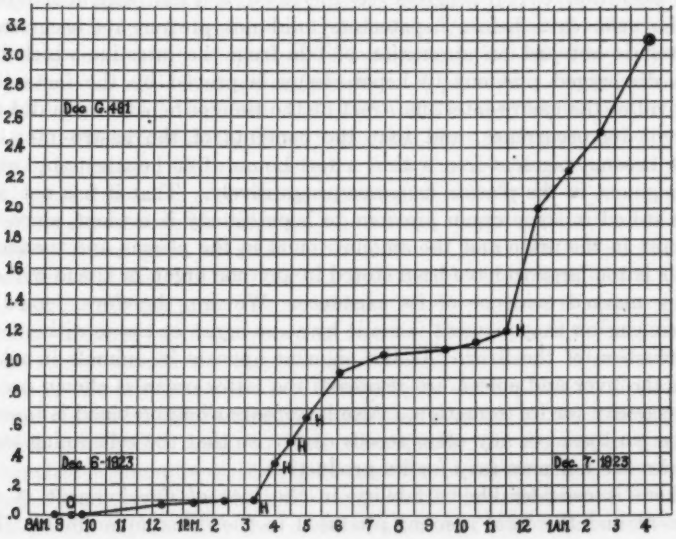


Fig. 3. Curve of bilirubin accumulation in the blood following total removal of the liver, *O*, and the subsequent injection of hemoglobin, *H*. Note the increase in the bilirubin content of the blood following the injection of hemoglobin.

animals. This variation has seemed to bear some relation to the condition of the animal with regard to the amount of fat, the amount of free blood in the peritoneal cavity, and the activity of the animal after operation.

Discussion. A yellow color of the urine, plasma and fatty tissue has developed consistently in all hepatectomized animals living more than six hours. The yellow color of the urine is so pronounced and typical that one never fails to notice it, or hesitates to pronounce the animal jaundiced. Since all the tests are positive, we are forced to conclude, so far as present day chemical methods will allow, that the pigment is bilirubin.

We have considered four possible sites of origin of the pigment: 1, squeezed from the liver at the time of operation; 2, absorbed from the bile present in the intestine previous to operation; 3, stored in some organ previous to operation; and 4, made by some tissue. It should be emphasized that in these experiments there can be no question concerning the possibility of remaining liver tissue having caused the production of the pigment, since all of the liver was removed.

That the pigment was not squeezed from the liver at the time of operation is proved by the facts that it was not found in the plasma immediately after operation; that it progressively increased, and that careful clamping of the vena cava before hepatectomy did not prevent its occurrence. That it is not just an absorption of bile from the intestine which had been discharged previous to hepatectomy is proved by the facts that removal of the gastro-intestinal tract at the time of hepatectomy did not prevent its occurrence, and that the contents of the gastro-intestinal tract become pigment-free within a few hours after hepatectomy while the pigment in the plasma increases progressively. That it has not just been stored in some organ previous to hepatectomy is evident, since the amount in the urine and the amount found in the plasma and tissues at death is too large to have been stored in any one organ or tissue without giving marked evidence of its existence. We are, therefore, forced to the conclusion that pigment is made by some organ or tissue without the intervention of the liver. As the pigment appears in animals in which both the liver and spleen are removed, the latter organ is shown not to be necessary for its formation. Also it appears when no blood is present in the peritoneal or any other serous cavity, so that the cells lining these cavities are not necessary for its production.

There is considerable variability in the rate of development of the pigment and the total amount produced in the different animals. The pigment appears much sooner after operation in some animals than in others, while a much greater amount of pigment may be formed in some animals than in others. Definite reasons for the individual variability

in pigment production have not been ascertained, although suggestive observations have been made. In some respects this variability may be more apparent than real. For instance, it seems to develop more slowly in the urine and plasma in fat animals than in those in which subcutaneous fat is lacking. This is possibly due to the fact that the pigment accumulates in the fat. The development of pigment seems to be more rapid and in larger total amounts in very active animals than in quiet ones; this may be associated with the increased blood destruction which follows exercise. The pigment also seems to develop in larger amounts in animals in which a small amount of intraperitoneal hemorrhage has occurred.

From what we know with regard to the origin of bilirubin, or similar pigments, there would seem to be but one possible source of this pigment, the hemoglobin. It should be emphasized that most of our observations have been made on hepatectomized animals in which no effort was made to increase blood destruction, or to add hemoglobin to the circulating blood. In one small series of animals, laked blood, either the animal's own, or obtained from another dog, was injected intravenously. When the laked blood was injected immediately after hepatectomy, the rate of formation of the pigment did not seem to be increased over the rate at which it formed in animals in which hemoglobin was not injected. However, if the laked blood was not injected until after a definite amount of pigment had already been formed, the rate of formation of bilirubin was definitely accelerated. Injections of small amounts of laked blood seemed to produce as rapid and great formation of pigment as large amounts. It should be noted that both the liver and kidneys excrete hemoglobin when it is in excess in the blood; also that they both excrete bilirubin; that an increase in bilirubin excretion from the liver does not take place until some time after an increase of hemoglobin in the blood, and that in many species of animals bilirubin is a normal constituent of the blood.

One problem gave us great concern for a time: when the common bile duct of a dog is obstructed, a demonstrable pigment in the blood does not appear for many hours, whereas when the liver is removed, the pigment appears within a very few hours. This seemed to imply that hepatectomy caused an increase in blood destruction. However, the counts of the erythrocytes after hepatectomy did not admit of such an increase in blood destruction, because the number of erythrocytes remained practically normal. This discrepancy in the appearance of demonstrable amounts of the pigment in the blood stream of hepatectomized animals, and those with the common bile duct obstructed, has been satisfactorily explained by the results of a series of experiments on normal dogs in which the gallbladder was removed simultaneously with obstruction of the common bile duct. In such animals the pigment appeared in the blood at approximately the same rate as in hepatectomized animals.

Furthermore a hepatectomized animal presents the same general appearance with regard to pigment in urine, plasma and fat as an animal from which the gallbladder has been removed and the common bile duct occluded.

The results of our experiments show conclusively that there accumulates in the body after removal of the liver a pigment which in all its characteristics appears to be bilirubin. This proves definitely that at least a portion of the bile pigment can be produced outside the liver. While in our experiments the rate of formation of bilirubin after hepactectomy has been less than the rate of excretion of bile pigment from the normal liver, it has been formed at a rate which would justify the question with regard to how much of the bile pigment normally is formed by the liver and how much formed elsewhere. We can definitely state that to a considerable extent the liver must be considered an excretory organ so far as bile pigment is concerned.

We are unable to determine where the pigment is formed. Its formation is not dependent on any of the intra-abdominal organs because it accumulates at approximately the same rate after they have all been ablated. It is possible that endothelial cells throughout the body have the ability to form bile pigment. The logical site for both the destruction of worn-out, functionless erythrocytes, and the disintegration of hemoglobin would be at the point where the greatest functional stress is placed on the erythrocytes, in the capillaries.

SUMMARY

A yellow pigment accumulates in the urine plasma and fatty tissues following total removal of the liver in the dog. The pigment accumulates progressively. It is usually noted in the first specimen of urine secreted after hepatectomy. In from three to six hours after the liver is removed, the plasma becomes yellow tinged. In animals that live sixteen hours or more, the sclera becomes tinged with yellow. At necropsy all the fatty tissue of the body is a dirty yellow color. This yellow pigment gives a positive reaction for bilirubin. The formation of this pigment is not dependent on any of the intra-abdominal organs. Its rate of development, under certain conditions, is accelerated by the intravenous injection of laked blood. The liver is not essential for the formation of bilirubin. It would seem highly probable that normally a high percentage of the bile pigment excreted by the liver is of extrahepatic origin.

PROTOCOLS. *Experiment 177–20:* This is one of our earlier experiments on the effect of hepatectomy, before we were aware that the administration of glucose was of great value in maintaining the life of the hepatectomized animal. The experiment, which is typical of a large series, proved to us that a pigment developed after hepatectomy which gave a positive reaction for bile pigment.

Dog D548 was a black and white male, adult bull terrier, in good condition, weighing 13.4 kgm. March 12, 1920, a reverse Eck fistula was performed. April 13, the animal had recovered from the Eck fistula operation and remained in good condition. The portal vein was ligated on this date. The intestines remained pink after ligation. The site of operation was in excellent condition, but a few adhesions were present. May 24, the animal had recovered from the ligation and was in excellent condition. He was kept on a regular diet until May 18, when he was put on a milk diet for two days and then fasted for two days. The liver was removed May 22.

At 9:00 a.m. the pulse was 140, the respiration 32, and the temperature 39.4°. At 10:55 a.m. the pulse was 120, the respiration 32, and the temperature 39.3°. Between 10:55 a.m. and 11:00 a.m., 30 cc. of blood were removed from the jugular vein. The plasma was clear, and a chloroform extract was negative to the nitric acid test for bile. At 11:00 a.m. the animal was placed in the etherizing cabinet, etherized, placed on the table and intubated. At 11:10 a.m. the first incision was made; at 11:25 a.m. the liver, weighing 372 grams, had been removed. At 11:35 a.m. the abdominal wound had been completely repaired. At 11:37 a.m. urine was removed from the bladder. A nitric acid test showed that it was negative for bile. At 11:45 a.m. the animal was able to stand. At 12:00 a.m. the pulse was 225, the respiration 65, and the temperature 38.2°. At 2:00 p.m. the animal appeared normal; the pulse was 200, the respiration 68 and the temperature 38.4°. At 3:00 p.m. the pulse was 200, the respiration 60 and the temperature 38.7°. At this time the animal was demonstrated to the members of the Mid-West Interurban Clinic. It appeared to be normal, stood, walked, drank water, and responded when spoken to, and did not differ from other animals following an operation of similar length. At 5:00 p.m. the pulse was 200, the respiration 60, and the temperature 39°. At 5:45 p.m. blood was removed. The plasma was slightly yellow, and the chloroform extract gave a positive test for bile pigment (nitric acid). At 5:50 p.m., 70 cc. of urine were removed. It was bile colored and gave a very marked reaction for bile pigment (nitric acid). At this time the animal showed some evidence of muscular weakness; this developed quite quickly and in a few minutes, the animal was unable to stand. The pulse was 170, the respiration 58, and the temperature 39.1°. At 7:00 p.m. the animal's muscles began to twitch. The pulse was 180, the respiration 60, and the temperature 38.2°. The muscular twitchings became more marked, and developed into a few mild convulsions. At 7:30 p.m. the pulse was 120. It was impossible to take the respiration because of convulsive movements. The animal vomited, and died at 7:35 p.m. A chloroform extract of blood plasma at death was yellow and gave a positive nitric acid reaction for bile pigment. Thirty cubic centimeters of urine removed from the bladder also gave a positive nitric acid reaction for bile pigment.

Necropsy (234–20) was performed immediately after death. The site of the operation was in excellent condition. There was no hemorrhage in the peritoneal cavity. The intestines remained normal in color. The fatty tissue was a dirty yellow. The chloroform extract of the fat gave a positive reaction for bile pigment.

Experiment 246–23: This experiment demonstrates the development of bilirubin after removal of all the abdominal viscera. Also there was no blood in the peritoneal cavity.

Dog G192 was a male, adult mongrel, in good condition, and weighing 11.3 kgm. March 23, 1923, a reverse Eck fistula was performed. August 13, the animal weighed 12.5 kgm. He had remained in excellent condition since the Eck fistula operation. An attempt was made to ligate the portal vein, but the intestines became blue, indicating insufficient collateral circulation. The ligature was accordingly removed, and the portal vein about half occluded with a blood vessel suture. The intestines

remained pink following suturing. November 22, the animal remained in fair condition following the last operation. The following experiment was performed on this date:

The first blood specimen was taken and etherization begun at 10:20 a.m. The first incision was made at 10:30 a.m. and the entire gastro-intestinal tract, spleen, most of the pancreas, and kidneys were removed. The peritoneal cavity was filled with moist packs in order to keep the diaphragm in proper condition. The entire liver, which weighed 418 grams, was removed at 10:45 a.m. The animal weighed approximately 11.0 kgm. post-operatively. At 11:00 a.m., immediately after operation, 1 gram of 50 per cent glucose was injected for each kilogram of body weight. The animal recovered from the immediate effects of the operation and appeared normal for several hours.

At 2:00 p.m. the second blood specimen was taken, and 1 gram of 25 per cent glucose was injected for each kilogram of body weight. At 5:00 p.m. the third blood specimen was taken, and 0.5 grams of 25 per cent glucose was injected for each kilogram of body weight. The animal had been in excellent condition up to this time. At 7:25 p.m. the animal died suddenly; the fourth blood specimen was taken immediately after death. (Table 1.)

TABLE 1

Development and accumulation of pigment in the blood (Exper. 246–23)

BLOOD SPECIMEN NUMBER	TIME AFTER OPERATION	PLASMA YELLOW	ALCOHOL YELLOW	INDIRECT REACTION	ALCOHOL REACTION
1	0	0	0	0	0
2	3 hours, 15 minutes	±	+	±	±
3	6 hours, 15 minutes	+	+	0.08	+
4	8 hours, 40 minutes	+	+	0.10	+

Necropsy (679–23) was performed immediately. No free fluid was found in the peritoneal cavity, and practically no blood clots. The packs placed in the peritoneal cavity did not have a trace of blood stain. All fatty tissue was of a dirty yellow color, and a chloroform extract of the fat was yellow and gave a positive reaction for bile pigment. Grossly and microscopically the liver of this animal was normal.

Experiment 680–23: This experiment demonstrates the increase in bilirubin formation following the injection of hemoglobin in the hepatectomized animal.

Dog G491 was a male, adult mongrel, in good condition, weighing 24.2 kgm. August 22, 1923, a reverse Eck fistula was performed. October 11, the animal had remained in good condition following operation, and weighed 22.7 kgm. The abdomen was opened and the portal vein ligated. The intestines remained a fair pink color following ligation. December 6, the animal had remained in good condition following the last operation. On this date the following experiment was performed:

At 8:55 a.m. the first blood specimen was taken. At 9:00 a.m. the animal was etherized; at 9:10 a.m. the incision was made, and the liver was removed at 9:20 a.m. The ether was withdrawn at 9:40 a.m., and the last stitch was made at 9:45. The liver weighed 418 grams, and the animal weighed 20.7 kgm. postoperatively. At 9:45 a.m. the second blood specimen was taken, and 0.5 gram of 25 per cent glucose was injected for each kilogram of body weight. At 9:50 a.m. the animal voided urine. At 10:45 a.m. 0.25 gram of glucose was injected for each kilogram of body weight. At 11:45 a.m. this injection was repeated.

At 12:20 p.m. the third blood specimen was taken; 0.5 gram of 25 per cent glucose was injected for each kilogram of body weight. At 1:25 p.m. the fourth blood specimen was taken, and 0.25 gram of 25 per cent glucose injected for each kilogram of body weight. At 2:25 p.m. the fifth blood specimen was taken, and 0.25 gram of 25 per cent glucose injected for each kilogram of body weight. Hemoglobin was made from the fifth blood specimen as follows: 20 cc. in 10 cc. of 5 per cent sodium citrate, 0.9 per cent sodium chlorid. Centrifuged. Cells washed with 20 cc. 0.9 per cent sodium chlorid once. Ten cubic centimeters distilled water plus 5 drops ether added, and allowed to stand thirty minutes. Total volume — 20 cc. in 10 cc. mixed with 20 cc. 25 per cent glucose. At 3:25 p.m. the sixth blood specimen was taken, and 10 cc. of laked blood in 0.25 gram of glucose for each kilogram of body weight were injected. At 4:00 p.m. the seventh blood specimen was taken, and the above injection repeated. At 4:30 p.m. the eighth blood specimen was taken, and the injection repeated. At 4:45 p.m. the animal vomited. At 5:00 p.m. the ninth blood specimen was taken, and the injection repeated. At 6:05 p.m. the tenth blood specimen was taken, and 0.5 gram of glucose injected for each kilogram of body weight. At 7:30 p.m. the eleventh blood specimen was taken, and the above injection repeated. At 9:00 p.m. the animal voided 125 cc. of urine. At 9:30 p.m. the twelfth blood specimen was taken, and 0.5 gram of 25 per cent glucose was injected for each kilogram of body weight. The animal was in fair condition, able to walk normally, but preferring to lie quietly. He responded to call. At 10:30 p.m. thirteenth blood specimen was taken, and blood was taken for hemoglobin. One-fourth gram of 25 per cent glucose was injected for each kilogram of body weight. At 11:30 p.m. the fourteenth blood specimen was taken, and 15 cc. of laked blood was injected as before in 0.25 gram of glucose for each kilogram of body weight. At 12:30 a.m. the fifteenth blood specimen was taken, and 0.5 gram of 25 per cent glucose injected for each kilogram of body weight. At 1:30 a.m. the sixteenth blood specimen was taken, and 0.25 gram of 25 per cent glucose injected for each kilogram of body weight. At 2:30 a.m. the seventeenth blood specimen was taken, and 0.5 gram of 25 per cent glucose injected for each kilogram of body weight. At 4:00 a.m. the animal was in good condition. He had remained quiet for the last few hours. At 4:25 a.m. the animal was found dead, and the eighteenth blood specimen was taken.

Necropsy (680–23) was performed at once. The third specimen of urine, 48 cc., was obtained. The peritoneal cavity contained a small amount of blood-stained fluid, but not enough to complicate the results of this experiment in any way. All the fatty tissue was a dirty yellow. A chloroform extract of this fatty tissue was yellow, and gave a positive test for bile pigment. The scleras showed a faint, but definite jaundice. (Table 2.)

Experiment 919–23: In this experiment the formation of the pigment in the plasma was followed quantitatively, and the life of the hepatectomized animal was maintained until the plasma became definitely jaundiced. It is representative of a large series of experiments.

Dog G671 was a female, adult, black and white mongrel, in good condition, and weighing 15.9 kgm. November 14, 1923, a reverse Eck fistula was made. January 15, 1924, the animal weighed 15.8 kgm. The animal had remained in good condition after the operation. The abdomen was opened and the portal vein ligated. The intestines remained a fair color following ligation, but the animal did not seem to have a good chance of surviving. Accordingly the spleen was also removed in order to cut down the amount of blood passing into the portal vein. March 13, 1924, the animal recovered and remained in good condition. On this date the following experiment was performed:

At 8:50 a.m. the first blood specimen was taken. The bladder was empty. The animal was etherized at 9:00 a.m. The incision was made at 9:05 a.m., and the liver was removed at 9:15 a.m. Ether was withdrawn at 9:30 and the last stitch was taken at 9:32 a.m. The animal weighed 13.2 kgm. following the operation, and the liver weighed 339 grams. At 9:30 a.m. 27 cc. of 10 per cent glucose in 0.8 per cent of sodium chlorid were injected intravenously. At 10:15 a.m. 27 cc. of 10 per cent glucose in 0.8 per cent sodium chlorid were injected intravenously. The animal was in good condition, although rather quiet. At 11:15 a.m. 27 cc. of 10 per cent glucose

TABLE 2

Development of the pigment (Exper. 680–23)

BLOOD SPECIMEN NUMBER.	TIME AFTER OPERATION	PLASMA YELLOW	HEMOGLOBIN PRESENT	ALCOHOL YELLOW	INDIRECT REACTION	ALCOHOL REACTION
2	25 minutes	0	0	0	0	0
3	3 hours	+	0	+	0.07	+
4	4 hours, 5 minutes	+	0	+	0.08	+
5	5 hours, 5 minutes	+	0	+	0.09	+
6	6 hours, 5 minutes	+	0	+	0.10	+
7	6 hours, 40 minutes	++	+	++	0.33	0.32
8	7 hours, 10 minutes	++	+	++	0.47	0.45
9	7 hours, 40 minutes	++	++	++	0.62	0.60
10	8 hours, 45 minutes	++	++	++	0.92	0.90
11	10 hours, 10 minutes	++	+	++	1.05	1.02
12	12 hours, 10 minutes	++	0	++	1.08	1.10
13	13 hours, 10 minutes	++	0	++	1.12	1.13
14	14 hours, 10 minutes	++	0	++	1.12	1.30
15	15 hours, 10 minutes	+++	++	+++	2.00	2.00
16	16 hours, 10 minutes	+++	+	+++	2.25	2.20
17	17 hours, 10 minutes	+++	+	+++	2.50	2.50
18	19 hours, 5 minutes	+++	+	+++	3.10	3.0

Notes: Yellow color of plasma seems to develop with a definite progressive increase, and with some sharp increases after hemoglobin injections. Yellow alcohol was increasingly colored. Marked increase is denoted by an additional plus.

Blood specimen no. 10, slightly biphasic. Blood specimen no. 18, rapidly biphasic.

Urine no. 1, negative for bile pigment.

Urine no. 2, strongly positive for bile pigment.

Urine no. 3, strongly positive for bile pigment.

were injected intravenously. At 11.50 a.m. the second blood specimen was taken. The animal was catheterized, and 34 cc. of urine were obtained. Twenty-seven cubic centimeters of 10 per cent glucose were injected intravenously.

At 1:00 p.m. 27 cc. of 10 per cent glucose were injected intravenously. At 2:00 p.m. this injection was repeated. At 2:50 p.m. the third blood specimen was taken. The animal was catheterized and 60 cc. of urine obtained. The same injection of glucose was repeated at this time, and again at 4:15 p.m. and at 5:20 p.m. At 5:50 p.m. a third specimen of urine was taken, 38 cc. being obtained. The fourth blood specimen was taken, and 27 cc. of 10 per cent of glucose were injected. The animal was in good condition. At 7:10 p.m., and again at 8:00 p.m. the injection was repeated. The animal was in good condition. At 8:50 p.m.

the fifth blood specimen was taken. The animal was catheterized, and 36 cc. of urine obtained. The injection of glucose was repeated in the same dosage as before. The animal was in good condition. At 9:45 p.m. the injection of glucose was repeated. The animal felt warm to the touch. The rectal temperature was 39.9. There was a definite yellow tinge to the scleras. The animal vomited a slight amount of fluid. At 10:45 p.m. 27 cc. of 10 per cent glucose were injected. Some of this infiltrated. The vein was exposed, and another 20 cc. of glucose were injected at 11 p.m. The rectal temperature was 39.7°. At 11:50 p.m. 21 cc. of urine were catheterized. The sixth blood specimen was taken, and 40 cc. of 10 per cent glucose were injected.

At 12:40 a.m. 30 cc. of 10 per cent glucose were injected. The animal was in good condition. At 1:55 a.m. 40 cc. of 10 per cent glucose were injected. The animal was still in good condition, but had retched considerably during the last hour. At 2:50 a.m. the animal was in fair condition, but was retching considerably. The urine was catheterized, and the seventh blood

TABLE 3

Development and progressive increase of pigment in the blood (Exper. 919–23)

BLOOD SPECIMEN NUMBER	TIME AFTER OPERATION	PIGMENT		
		Plasma yellow	Alcohol yellow	Indirect reaction
1	0	0	0	0
2	2 hours, 30 minutes	+	±	0
3	5 hours, 30 minutes	+	+	+
4	8 hours, 30 minutes	+	+	+
5	11 hours, 30 minutes	++	+	0.4
6	14 hours, 30 minutes	++	++	0.5
7	17 hours, 30 minutes	++	++	0.7
8	20 hours, 30 minutes	+++	+++	0.8
9	23 hours, 30 minutes	+++	+++	0.9
10	25 hours, 30 minutes	+++	+++	0.9

Note: The second specimen of urine after operation and all subsequent specimens showed a considerable amount of bile pigment, which was fairly constant.

specimen taken. Forty cubic centimeters of 10 per cent glucose were injected. At 4:00 a.m. the animal became restless and retched constantly. Forty cubic centimeters of 10 per cent glucose were injected. At 5:20 a.m. the animal was still restless and retching; otherwise, it was in very good condition. It stood up in its cage, and wagged its tail. Forty cubic centimeters of 10 per cent glucose were injected. At 5:50 a.m. the animal was still restless, but in good condition. Fourteen and five-tenths cubic centimeters of urine were catheterized, and the eighth blood specimen taken. Thirty cubic centimeters of 10 per cent glucose solution were injected. At 6:50 a.m. the animal showed the first signs of the second stage; it was breathing hard and kept its nose against the cage. It was given 30 cc. of 10 per cent glucose. The rectal temperature was 40.4°. At 7:45 a.m. the animal seemed to be going into coma, but could still walk around and wag its tail. Forty cubic centimeters of 10 per cent glucose solution were injected. At 8:50 a.m. the ninth blood specimen was taken. Five grams of glucose, 1 gram of sodium sulphate and 0.5 gram of bicarbonate in 100 cc. were injected. Eighteen cubic centi-

meters of urine were catheterized. The rectal temperature was 41.2°. The animal was definitely comatose; the pulse and respiration were regular, but somewhat rapid. At 9:40 a.m. 150 cc. of 10 per cent glucose were injected. Six cubic centimeters of urine were catheterized. At 10:40 a.m. 20 cc. of 50 per cent glucose were injected. The animal died at 10:50 a.m. Seventeen cubic centimeters of urine were taken from the bladder and added to the 6 cc. taken at 9:40. The tenth blood specimen was taken from the heart.

Necropsy (189–24) was performed immediately after death. The scleras and mucous membranes were definitely yellow, and the fat was very yellow. About 200 cc. of blood-stained fluid were found in the peritoneal cavity. (Table 3.)

BIBLIOGRAPHY

(1) Adler and Meyer: Klin. Wochenschr., 1923, ii, 258.
(2) Aschoff: München. med. Wochenschr., 1922, ii (N. S.), 1352.
(3) Bickel: Deutsch. med. Wochenschr., 1923, xlix, 140.
Brown, McMaster and Rous: Journ. Exper. Med., 1923, xxxvii, 699.
(4) Brugsch and Yoshimoto: Zeitschr. f. exper. Path. u. Therap., 1910–11, viii, 639; Brugsch and Kawashima; Ibid., 645.
(5) Brulé: Recherches sur les Ictères, Paris, 1922, xiii, 280.
(6) Feigl and Querner: Zeitschr. f. d. gesammt. exper. Med., 1919, ix, 153.
(7) Froin: Gaz. d. hôp., Paris, 1906, lxxix, 75, 1907, lxxx, 1539, Compt. rend. Soc. de biol., 1906, lx, 121.
(8) Gilbert: Quoted by Brulé.
(9) Gmelin: Hawk's Practical physiological chemistry. Philadelphia, 7th ed., 1921, 210.
(10) Herzfeld: Deutsch. Arch. f klin. Med., 1922, 139, 306.
(11) Huppert: In Cole's Practical physiological chemistry, 6th ed., 1920, 268.
(12) Jones and Jones: Arch. Int. Med., 1922, xxix, 669.
(13) Kunde: De hepatis ranarum exstirpatione. Berlin, 1850.
(14) Lepehne: Ergebn. d. inn. Med. u. Kinderh., 1921, xx, 221.
(15) Lepehne: München. med. Wochenschr., 1919, i (N. S.), 619.
(16) McMaster, Broun and Rous: Journ. Exper. Med., 1923, xxxvii, 395.
(17) McNee: Quart. Journ. Med., 1923, xvi, 390.
(18) McNee: Med. Klin., 1913, ii, 1125.
(19) McNee and Prusik: Journ. Path. and Bact., 1924, xxvii, 1924.
(20a) Mann: Amer. Journ. Med. Sci., 1921, clxi, 37.
(20b) Mann and Magath: Trans. Sect. Path. and Physiol., Amer. Med Assoc., 1921, 29.
(21) Mann and Magath: This Journal, 1921, lv, 285; Arch. Int. Med., 1922, xxx, 73.
(22) Minkowski and Maunyn: Arch. exper. Path. u. Pharm., 1886, xxi, 1.
(23) Minkowski and Stern: Ueber die normale Bildungsstätte des Gallenfarbstoffes, Dissertation, Königsberg, 1885.
(24) Moleschott: Arch. f. Physiol. Heilk., 1852, xi, 479.
(25) Müller: Handbuch der Physiologie des Menschen für Vorlesungen, 4 aufl., 1844.
(26a) Rich: Bull. Johns Hopkins Hosp., 1923, xxxiv, 321.
(26b) Editorial, Journ. Amer. Med. Assoc., 1923, lxxxi, 2116.
(26c) Editorial, Journ. Lab. and Clin. Med., 1924, ix, 282.
(27) Roger: Physiologie normale et pathologique du foie. Paris, 1922, 398 pp.
(28) Rosenberg: Hawk's Practical physiological chemistry, 7th ed., 1921, 448.
(29) Rosenthal and Meier: Arch. f. exper. Path. u. Pharm., 1921, xci, 246.

(30) ROSENTHAL AND MELCHIOR: Arch. f. exper. Path. u. Pharm., 1922, xciv, 28.
(31) SCHILLING: Berlin klin. Wochenschr., 1921, lviii, 881.
(32) STADELMANN: Der Icterus und seine verschiedenen Formen. Stuttgart, 1891.
(33) TARCHANOFF: Pflüger's Arch., 1874, ix, 53, 329.
(34) VAN DEN BERGH: Der Gallenfarbstoff im Blute. Leiden, 1918, 111 pp.
(35) VAN DEN BERGH: Presse méd., 1921, xxix, 441.
(36) VIRCHOW: Arch. f. path. Anat. u. Physiol., 1847, i, 379, 407.
(37) VÖGEL: Quoted by STADELMANN.
(38) WHIPPLE AND HOOPER: Journ. Exper. Med., 1913, xvii, 593.
(39) WHIPPLE AND HOOPER: Journ. Exper. Med., 1913, xvii, 612.
(40) WHIPPLE AND HOOPER: Journ. Exper. Med., 1916, xxiii, 139.

CIRCULATORY RESPONSES TO EXERCISE IN MAN AND THEIR BEARING ON THE QUESTION OF DIASTOLIC HEART TONE

H. L. WHITE

From the Physiological Department of Washington University, St. Louis

Received for publication April 23, 1924

The question of heart muscle tone has long been a subject of controversy. The idea that the heart muscle possesses tone, in the sense that diastolic relaxation may not be complete even though ample time is allowed for relaxation, was first proposed by Gaskell (1) on the basis of perfusion experiments on the frog's ventricle. This view is no longer generally held by physiologists. The present view may be summarized by the statement that the conception of diastolic tone seems to prevail among clinical writers and is shared by some physiologists, notably Yandell Henderson (2), (3), (4). Henderson holds not only that diastolic relaxation may be incomplete but that the extent of this incompleteness is variable. Yamada (5), working with dogs, aseptically removed the heart from the pericardial sac and allowed the animals to recover. Some of the animals were then subjected to as vigorous exercise as they would perform and after a few days the heart was removed and examined microscopically. In no case was hemorrhage in the heart wall discovered. Three of the animals showed slight subserous ecchymoses. Yamada states "dass diese Ekchymose pathologischer Natur und nicht Folge des mechanischen Prozesses war," although the evidence for this statement is not given. Henderson (4) states that "Yamada. . . . found that animals in which the pericardium had been cut open aseptically, the chest closed again and the subjects restored to normal health were able to make vigorous physical exertion with no injury to the heart. He offers the very probable explanation that during increased rapidity of beat, the corresponding increase in tonus prevents the ventricles from dilating so far." We would point out that *a*, the only criterion of the heart's condition was the presence or absence of myocardial hemorrhage. The absence of this was taken to mean that the heart was uninjured and undilated. No venous pressure determinations were made nor observations on the size of the heart during life and no measurements of the heart post mortem are given. *b*, The statement that "the subjects were able to perform vigorous physical exertion" is not consistent with Yamada's

statement that "es sehr schwer war, die Tiere für eine längere Zeit schnell laufen zu machen. Nach kurzem Laufe liebten die Tiere eine Zeit lang auf dem Boden sitzen zu bleiben, obgleich man sie zu weiterem Laufen anzutreiben suchte." Obviously the animals were far from being in a normal condition. *c*, The writer has been unable to find in Yamada's paper his "very probable explanation that during increased rapidity of heart beat, the corresponding increase of tonus prevents the ventricles from dilating so far." The reference is presumably to Yamada's statement, "Aus den obengenannten Ergebnissen kann man den Schluss ziehen, dass die Extensionskraft des zuströmenden Blutes (Venendruck) bei einer durch Muskelanstrengungen bzw. Medikamente beschleunigten Blutzirkulation nicht so gross wird, dass Blutung in der Herzwand auftritt. In solchen Zuständen schlägt das Herz meist sehr schnell, die Kammern werden besser entleert, und infolgedessen steigt der Venendruck trotz einer vermehrten Blutzufuhr nicht stark an. Das Herz wird dabei nicht dilatiert sein." The writer interprets this as meaning that Yamada's explanation of the failure of the heart to dilate is the increased heart rate, which results in the removal of blood from the venous side at such a rate that the venous pressure is not allowed to rise. This is an entirely different conception from that of an increased heart tone. It is hardly worth while however for us to concern ourselves here as to the exact nature of Yamada's explanation of the heart's failure to dilate, for the simple reason that he fails to show that it does not dilate. The writer would disagree then with Henderson in his statement that these experiments may be cited as evidence of ventricular tone.

On the other hand, some of the opponents of the heart tone idea have been no more careful in their interpretations. Bainbridge's statement (6) that "Gaskell's observations (1) do not necessarily imply the existence of diastolic tone, since they can be equally well explained by assuming that alkali prolongs the systolic phase and cuts short the period of diastole, whereas acid has the converse action," raises the question as to what is meant by tone. It seems to the writer that Bainbridge has merely made a rhetorical distinction. There are numerous other bits of evidence in favor of diastolic tone, particularly in the excised heart, which have been too lightly dismissed by those who do not subscribe to the idea. Every laboratory worker has seen tone waves occurring in a series of contractions of a strip of terrapin's auricle. The now well-established finding of a positive electrical change on vagus stimulation might also be considered evidence of a preëxisting tone. While none of these findings has proved the existence of heart tone in the normal intact animal, they cannot be disregarded. It is quite possible that the degree of tone, and even its very presence or absence, varies in different species. So far as the writer has been able to discover, the experiments reported in

this paper come more nearly to constituting proof of diastolic heart tone in the normal human subject than does any work previously reported.

This work was suggested by a statement appearing in the literature which appeared to the writer to be a direct contradiction to the idea that the heart does not possess a diastolic tone. This statement apparently has never been questioned and the fact that it contradicts the view held by most physiologists that (6) "The balance of evidence, therefore, is against the existence of diastolic tone, even in the normal heart, and the conclusion appears unavoidable that the heart muscle is normally completely relaxed during diastole. . . .", appears never to have been recognized. The statement referred to is that of Hooker (7) and of Schneider, Cheley and Cisco (8) that the venous pressure is elevated during exercise *and does not return to normal for several minutes after the cessation of exercise.* The following considerations will make clear the mutual contradiction of this statement and that of Bainbridge (6).

We may state here that our interpretation of the venous pressure findings must depend upon our ideas as to the changes which the stroke volume of the heart undergoes during and after exercise. As is well known, there are two conflicting views on this point. Krogh and Lindhard (9), (10) and Lindhard (11), (12), using the nitrous oxide method, find that the stroke volume of the heart increases markedly during exercise. On the other hand, Yandell Henderson (4) finds the stroke volume constant during exercise and rest except in so far as excessive heart rates may actually diminish the stroke volume due to inadequate time allowed for diastolic filling. Henderson (4) quotes Boothby (13) as finding a considerable increase in stroke volume during exercise. An inspection of his data, however, shows only a relatively small increase. Douglas and Haldane (14), using the method of analyzing the alveolar air when it is in equilibrium with venous and with arterial blood, find in some subjects a constant stroke volume during exercise and rest, in others an increase during exercise.

The x-ray evidence as to the changes in the diastolic volume of the heart during and after exercise is no less contradictory. Bingel (15), de la Camp (16), Lennhoff and Levy-Dorn (17), Mendel and Selig (18), Hoffmann (19), Kienbock, Selig and Beck (20), found no change or a slight decrease in the heart shadow immediately after exercise. Bainbridge (21) objects that this type of experiment throws no light on the behavior of the heart *during* exercise on the grounds that the heart shadow might have been increased during the exercise but this increase failed to be seen when the observations were made even a few seconds after cessation of exercise. He states that the heart might be expected to diminish in size almost instantly after the exercise stops, its diastolic volume falling to or below normal within three or four seconds. He quotes the work of

Nicolai and Zuntz (22) in support of this idea. These workers employed a subject working on a treadmill. The plate on which the picture was to be taken was fastened to the subject's chest and the picture was taken as soon as possible after the exercise stopped. They found some increase in the heart shadow "during" exercise (which means within one or two seconds after exercise); this is followed by a decrease which may be evident within three seconds. They calculate that the stroke volume of one ventricle may increase from 60 cc. during rest to 150 cc. during work. It must be noted, however, that even in these experiments the observations were not made strictly during work. The subject was in the erect position. Bainbridge fails to note that Moritz (23)[1] observed the heart *during* exercise and found an unmistakable decrease in the heart shadow. The subject lay on a table and exercised by holding the arms or legs extended to the point of fatigue, the pictures being made during the actual act of exercise. Failure to obtain an increase in the heart shadow with this static form of exercise does not, however, justify one in assuming that the same circulatory conditions obtain as in the usual forms of exercise. In Moritz' experiments the pumping action of the skeletal muscles was absent. One could hardly expect an increased venous return and it is therefore not surprising that heart shadow failed to increase or even decreased. Bruns (26) observed 46 normal human subjects in the erect position, the exercise consisting of alternate flexion and extension of one leg. X-ray observations during the very act of exercise showed that even during exercise severe enough to cause a rise in pulse rate of from 10 to 50 per minute and in blood pressure (presumably systolic) of from 10 to 40 mm. Hg there was never any significant increase in the heart shadow. He found sometimes a decrease, sometimes no change; any increase was never more than would correspond to an increase of 3 to 5 cc. in heart volume, which must be within the limits of experimental error. He checked up on the accuracy of the method by demonstrating a marked decrease of heart shadow in the Valsalva experiment. Meek and Eyster (27) in x-ray observations on 17 normal individuals found an increased diastolic size during exercise in 10 subjects and a decreased in 7. In 11 subjects the systolic size showed a decrease during exercise and in 6 an increase. The output per beat at rest averaged 79.4 cc., during exercise 94 cc. In 3 subjects there was an actual decreased output per beat during exercise. In certain experiments there was an increased stroke volume accompanying a decrease in diastolic size, indicating a more complete systolic emptying.

It has been necessary to digress from our theme in order to consider this question of the behavior of the stroke volume during and after ex-

[1] This is presumably the work which Henderson (24) credits to Dietlen (25). This work is referred to by Dietlen in his review but was done by Moritz.

ercise. It will be noted that we have considered only such work on this subject as has been done on the human subject. The voluminous literature reporting work done with the heart-lung preparation (28) is not even touched upon. Having interjected this fragmentary review of the literature on the subject the writer will leave the reader to decide for himself which view is supported by the better evidence. It is not the place to enter here into a discussion of this problem. We would merely call attention to the bearing which it has on our consideration of the relation of Hooker's and Schneider, Cheley and Cisco's findings to the idea of diastolic tone.

We are now ready to consider how these findings contradict the conception that diastolic tone does not exist. It is generally conceded that the high rate of the venous return to the heart during exercise (and all workers agree that the return is increased) is kept up largely by the pumping action of the skeletal muscles. Since this pumping action ceases immediately on the cessation of exercise we should expect the inflow to and consequently the volume output of the heart per minute to fall rapidly on the cessation of exercise. Whether or not the stroke volume, or output per beat also falls is, as we have just seen, still an open question. Since we do not propose to attempt to answer that question here the only course left open to us is to assume in turn that each theory is correct and then examine the consequences of such an assumption. Let us first accept the view that the stroke volume increases during exercise and falls rapidly after exercise (21), (12) and also that "the rate at which the heart fills varies directly with the pressure under which blood flows from the great veins into the heart (29)." This is the view apparently held by most physiologists today. We have then in the early phase of the post-exercise period a continued high venous pressure with a diastolic volume and stroke volume down to or below normal. These two conditions could exist simultaneously only if the diastolic tone is increased. We would point out that if we accept the view of a varying stroke volume, variations in the extent of filling must be assumed to be effected by variations in the rate of filling. Increase in extent of filling means increase in rate of filling, since, when the duration of the cardiac cycle is decreased, as it is during exercise when the increased extent of filling takes place, the curve of ventricular filling can rise higher than during rest only if its slope is steeper.

Let us now consider the view that the stroke volume is unchanged by exercise. We now have an increased venous pressure which does not result in any greater diastolic filling. The mere absence of an increased *extent* of diastolic filling does not, however, if we accept the view of a constant stroke volume, necessitate the assumption of an increased diastolic tone. The absence of an increased *rate* of filling would demand

our assumption of an increase in tone. As stated above, an increased extent of filling must mean an increased rate of filling. It does not follow, however, that a constant extent of filling necessarily means a constant rate of filling, i.e., a constancy of rate from one cardiac cycle to the next. If the resting venous pressure is sufficient to fill the heart adequately (in Krogh's sense, 30) any increase in venous pressure, unless so excessive as actually to stretch the muscle fibers, could not produce any further increase in extent of filling; it could only increase the rate. Now the advocates of Henderson's views by their very statement that the stroke volume is constant during rest and exercise say that the heart is adequately filled during rest. The point at issue is whether or not such an adequate filling necessitates our assumption of the operation of a varying diastolic tone. The writer feels that it is quite possible to accept the view of a constant stroke volume without accepting a varying diastolic tone. He is trying here merely to emphasize the conception that, although the failure of an increased *extent* of filling in the presence of an increased filling pressure means the failure of an increased *rate* of filling and therefore necessitates our assumption of an increase in tone *if we accept the view that the stroke volume during rest is smaller than during exercise*, this same failure of an increased extent of filling does not necessarily mean a failure of an increased rate of filling *if we accept the view that the heart is already adequately filled during rest.* Henderson has, of course, already appreciated these distinctions. He has recognized that the establishment of a mere constancy of extent of filling with a varying venous pressure is not enough to establish a variable tone and has therefore attemped to prove further that the rate of filling is also independent of the filling pressure. In an exposition of his thesis of the super-imposability of ventricular volume curves at varying heart rates and venous pressures, he says (31), "The rate and extent of ventricular filling is correspondingly independent of variations in the venous pressure (which is their distending force) so long at least as the supply of blood flowing from the veins into the auricles is not diminished, nor its pressure considerably lowered by hemorrhage or other abnormal condition." It must be pointed out, however, that the failure of an increase in venous pressure to effect an increase in rate of filling has been demonstrated only under quite artificial conditions and by a technique which many physiologists are not willing to accept as adequate to demonstrate any other than gross changes in the rate of filling. The superimposability of ventricular volume curves has never been demonstrated in the intact animal. When it has been demonstrated, and not until then, will the statement be justified that the rate of ventricular filling is unaffected by the filling pressure and not until then can Henderson's arguments on diastolic tone be accepted. To repeat, then, in the absence of reliable data on the rate

of ventricular filling the mere failure of an increased extent of filling with an increased venous pressure does not demand for its explanation an increase in diastolic tone, if we accept the view of a constant stroke volume, for we should not expect an increased filling pressure, unless it were excessive, to actually stretch the muscle fibers. We see, then, that if Hooker's and Schneider, Cheley and Cisco's finding of an increased venous pressure during and after exercise is correct, we must postulate a diastolic tone if we accept the view that the stroke volume changes during and after exercise but can draw no conclusions either for or against diastolic tone if we accept the view of a constant stroke volume, unless we are willing to accept as established Henderson's finding that the *rate* of ventricular filling is independent of the filling pressure, in which case an increase in diastolic tone during the period of increased venous pressure of exercise would of course be demanded. Strange to say, those authors (Bainbridge, Starling) who insist that the stroke volume changes on exercise are the very ones who most vigorously deny diastolic tone. It may be said, however, that Krogh, who holds that the stroke volume changes on exercise, admits the existence of diastolic tone (32). The work reported here is an investigation of certain circulatory responses to exercise with the hope of clearing this greatly confused issue.

Methods. Although the main question raised in this paper could perhaps be settled by an investigation of the venous pressure alone during and after exercise, it seemed advisable to follow at the same time the arterial pressure, pulse rate, respiratory rate and rate of work. Since some form of stationary exercise was essential to our purpose we naturally turned first to the bicycle ergometer. Shelves which could be fixed at any desired height were arranged for supporting the arm and hand on each side of the ergometer. The right hand, on which the venous pressure readings were made, was brought to the level of the costal angle. The shelf supporting the left arm and hand was brought to a convenient level and the cuff of the Erlanger sphygmomanometer applied over the left brachial artery. The writing point of the sphygmomanometer lever wrote on the drum of a long roll kymograph. The mercury manometer was provided with a float and writing point and so arranged that the pressure in the cuff was recorded on the drum under the record traced by the writing point of the sphygmomanometer lever. Respirations were recorded by a rubber dam pneumograph. Time was recorded in seconds; the pulse rate and respiratory rate could then be read from the sphygmomanometer and the pneumograph records.

It was soon found that while perfectly satisfactory records could be obtained with the subject at rest, he could not with this arrangement hold his arm quiet enough during exercise to obtain a satisfactory sphygmomanometer record. Very slight twitchings of the muscles under the

cuff render the record incapable of interpretation. The arm was next put in a suspension device in the hope that it could be held quiet during exercise. Even with this arrangement, however, there was enough movement when the exercise was anything above a minimum to invalidate the record. Supporting the arm in a rigid leather and steel brace made for putting up fractures of the upper part of the humerus did not give any better results. We finally gave up the hope of obtaining satisfactory arterial pressure records during exercise on the bicycle ergometer and set about arranging a device which would permit absolute quiet of the upper extremities during exercise of the lower extremities.

This apparatus, as completed, consists of a heavy wooden base, 180 × 60 cm., supporting a chair tilted back about 15°. An adjustable headrest is provided and the chair is comfortably padded with felt. A rigidly braced frame of gas pipe is erected over the chair and from a transverse bar 220 cm. above the base and 50 cm. in front of the chair hang two chains provided with hooks. The subject sits comfortably in the chair and extends his legs horizontally. His feet are in leather stirrups which are supported by the chains hanging from the high transverse bar. Cords are attached to the stirrups and by means of a system of pulleys, the last pair of which turn over a horizontal rod 100 cm. above the base and 120 cm. in front of the chair, a 5 kgm. weight is lifted at each extension of a lower extremity. The height of the lift is automatically recorded on the drum by the following arrangement. A slender rod is fastened at its upper end to a closely fitted horizontal bearing passing through a wooden block which is clamped to one of the uprights of the frame. This block can be fixed at any height on the upright. The lower end of the rod passes through a closely fitting ring rigidly fastened to a stirrup. The motion of the foot in the alternate extensions and flexions thus brings about a rotation of the bearing, which bears a lever provided with a writing point for tracing a record on the drum. By means of an appropriate calibration we can thus determine the number of kilogram meters of work per minute. It is of course recognized that these figures do not represent the absolute amount of work being done but a set of consecutive figures does represent the relative amounts of work done in the corresponding periods of time.

Our purpose is to follow the venous pressure, arterial pressure, pulse rate, respiratory rate and rate of work during and following a period of exercise. We may perhaps best describe a typical experiment. The procedure in all of the first set of experiments was identical with that described here. The subject sits quietly in the chair with his head on the headrest. His feet are placed in the stirrups, which are suspended by chains; the rod running to the bearing of the work recording lever is passed through the stirrup ring. The glass capsule for observing venous

pressure is sealed with collodion over a vein on the right hand and the level of this vein is made to coincide with the level of the costal angle. This is done by marking on the chest wall the level of the costal angle and adjusting the height of the shelf supporting the right hand until the level of the vein coincides with the level of the costal angle. This adjustment is made accurately with a carpenter's spirit level. The pneumograph is adjusted on the chest and the cuff of the sphygmomanometer on the left arm. The arrangement is such that the cuff is inflated by a compressed air tank, the inflation thus being a matter of only two or three seconds. Since the pressure in the cuff is recorded on the drum it is unnecessary to watch the sphygmomanometer record while it is being made in order to establish the pressures at which the systolic and diastolic criteria occur. One can have a laboratory technician manipulate the stopcocks for inflating and deflating the cuff and also start and stop the kymograph; the systolic and diastolic arterial pressures can be read at leisure on future examination of the record. The experimenter is thus left free to make the venous pressure determinations, which were carried out by means of an apparatus described in a recent article (33). Sweating of the skin under the glass capsule during exercise produces such an accumulation of droplets on the inner surface of the capsule that it is impossible to see the vein clearly enough to make accurate readings. This difficulty was circumvented by lightly coating the inner surface of the capsule with a solution of two parts of 95 per cent alcohol and one part of glycerine. This spreads out the moisture in a uniform film which does not interfere with the observations.

The subject is left to rest quietly for at least five minutes after all adjustments have been made. The drum is then started and three control determinations made at one-minute intervals on the resting subject. At a signal from the experimenter the subject then begins exercise. This continues for ten minutes and determinations of venous pressure and arterial pressure are made at one minute intervals throughout this period, the other data being automatically recorded. At the end of exercise the determinations are carried on for ten minutes at one-minute intervals. The results of one experiment are seen in table 1, a portion of a typical record in figure 1.

Results. The experiment described above was carried out in a series of eight subjects. In no case was the exercise heavy; if the type of exercise were being designated as light, moderate or heavy it would in all the cases in this series be designated as light; the highest average rate of work for the ten minute exercise period is 200 + kgm. M. per minute. The experiment outlined in table 1 is representative of the series. Lack of space forbids our presenting each experiment in detail. There are in certain experiments minor departures from the average results but these do not

invalidate the general conclusions, namely, that the pulse rate and respiratory rate rise immediately on exercise and fall rapidly on its cessation, the systolic arterial pressure rises somewhat on beginning exercise, which elevation is usually maintained during exercise; there is a rapid fall on cessation of exercise, sometimes to a subnormal level, whence it gradually returns toward normal. There is no consistent change in the diastolic arterial pressure except usually a very slight fall after exercise. The venous pressure usually rises somewhat (2 to 4 cm.) at beginning of exercise

TABLE 1

Subject He. Age 21, weight 77 kilos

VENOUS PRESSURE	ARTERIAL PRESSURE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	WORK PER MINUTE
cm. water	*mm.* Hg			*kgm. meters*
7	108–58	57	20	
7½	104–58	60	21	
7	108–56	60	26	
8½	112–58	78	30	161
10	126–62	80	25	170
10	118–57?	79	25	183
10½	115–54?	78	24	205
10	126–60	82	25	213
10	130–60?	86	28	218
10½	121–56?	86	26	226
10½	125–57	86	28	219
10½	121–54	80	31	207
10½	118–62?	84	25	207
7½	110–50	61	27	
7½	106–48	62	27	
7½	108–54?	66	23	
8	102–54	65	23	
7½	98–60	72	23	
7	105–?	67	24	
7	100–50?	63	25	
7½	100–?	62	22	
7	100–?	57	20	
7	103–54	54	19	

and falls immediately on its cessation, sometimes temporarily to a slightly subnormal level. Figure 2 presents graphically the average results of the eight experiments. The horizontal line indicates the normal resting level, the positions of the ordinates indicating the extent of rise or fall above or below this level. The respiratory rates and rates of work are omitted from the figure. The former behaves as one would expect with light exercise; the rate of work usually increased progressively up to the end of the work period.

XUM

Although tabulation of all the data of all the experiments would require too much space it has seemed advisable to present the venous pressure figures for all of the experiments, since these figures are the ones bearing most directly on the particular problem under discussion in this paper. These figures are seen in table 2. They designate the venous pressures in centimeters of water.

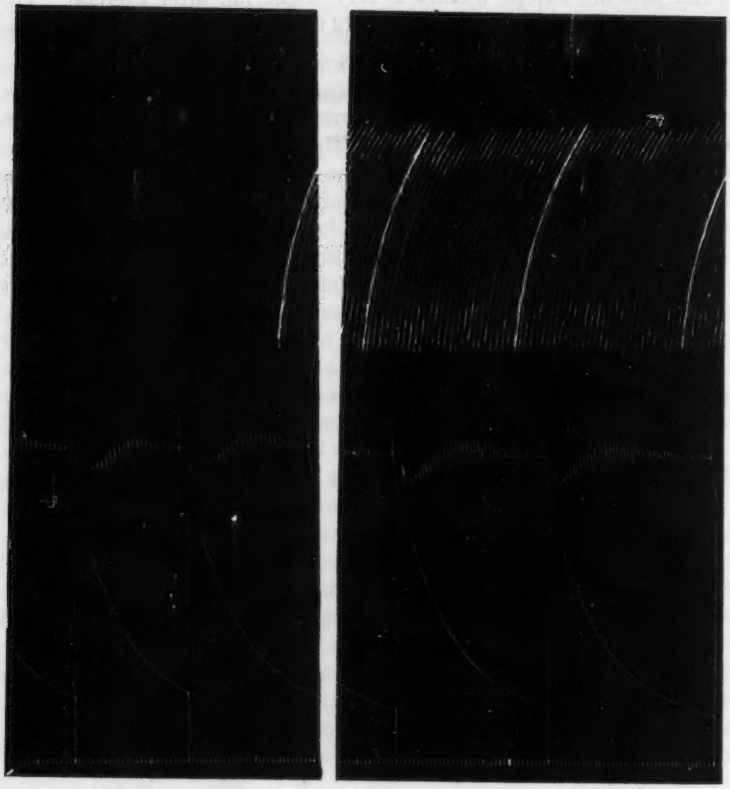


Fig. 1. Portions of record before and during work. Tracings from above down represent respirations, rate of work, sphygmomanometer record, pressure in cuff and time in seconds. Reduced one-half.

It will be noted that in no case is there a rise of venous pressure persisting for several minutes after exercise stops, as was reported by Hooker and by Schneider, Cheley and Cisco. We have not yet made an investigation as to whether or not the somewhat different technique employed (33)

can account for this discrepancy. We do find in this series of experiments, in common with Hooker and Schneider, Cheley and Cisco, an increased venous pressure during exercise. Let us now examine these findings with an eye to their bearing on the question of diastolic tone. We may first accept the view that the stroke volume of the heart increases during exercise and falls rapidly on its cessation. This would mean that an increased venous pressure results in an increased filling of the heart during exercise. This would not necessitate the assumption of any change in or even the existence of diastolic tone since it may be regarded as merely the effect of an increased filling pressure on a passively dilating heart.

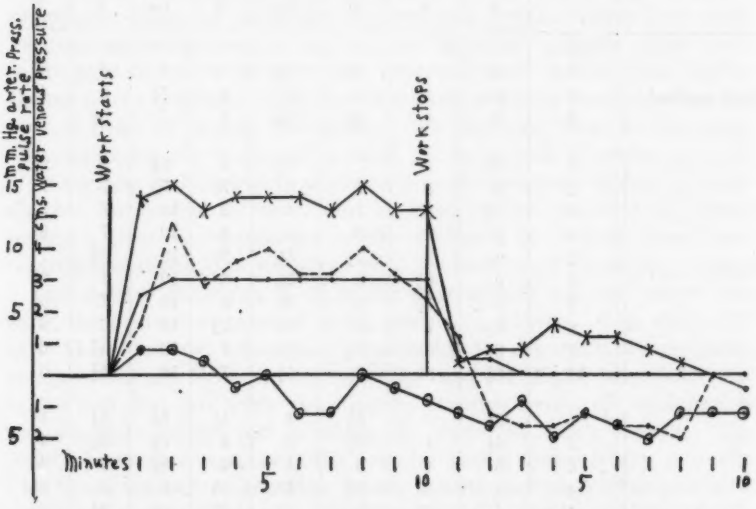


Fig. 2. Average results of the eight experiments in the first series, light work. —•—•—•— venous pressue, --•--•--•-- systolic arterial pressure, ⊙—⊙—⊙—⊙ diastolic arterial pressure, x—x—x— pulse rate.

Furthermore, the rapid fall of venous pressure occurring simultaneously with the post-exercise fall in stroke volume may be readily explained on the view that the heart fills passively in response to its filling pressure. In other words, in the light of these present findings we need not invoke a diastolic heart tone if we accept the idea of a varying stroke volume during and after exercise.

Let us now consider our interpretation of the findings on the view that the stroke volume is constant. We have now an increased filling pressure during exercise with no increase in diastolic filling. This, however, does not necessitate the invocation of an increased diastolic tone; as has been

pointed out above, an increased filling pressure (unless it were excessive) should not be expected to increase the extent of diastolic filling, merely its rate. Furthermore, the rapid fall to normal of venous pressure in the post-exercise period, accompanied by a maintenance of the constant stroke volume, might merely mean that the heart is adequately filled at the normal or resting venous pressure. At the risk of confusing the trend of his argument the writer feels constrained to pause here for an

TABLE 2

	TIME	SUBJECT							
		Z	M	D	Dv	H	He	F	T
	minutes								
Rest control	1	5	4½	5½	3½	4	7	3	4
	2	5	4½	5	3½	4	7½	3	4
	3	5	4	5½	3½	4	7	3½	4
Work	1	8½	7	7	5½	7	8½	6	6½
	2	9	8	7	6	7½	10	7	7
	3	9½	7½	7	6	8	10	7	7
	4	9	7½	8	6	8	10	7	6½
	5	9	7	7½	5½	7½	10	6½	7
	6	9	6½	8	5½	8	10	7	6½
	7	9	7½	8	5½	8	10	7	6½
	8	8½	7	8	5½	7½	10	6	6
	9	8½	6½	8½	5½	7	10	5½	6
	10	8½	6½	8	5½	7	10	5½	6
After work	1	5½	4	6½	4	4½	8½	3½	4
	2	5½	4	5½	4	4	8½	3½	4
	3	5	4	5	4	4	8½	3	4
	4	5	3½	5½	4	3½	8	3	3½
	5	5	3½	5½	3½	3½	8½	3½	3½
	6	5	3½	5	3½	3½	8	3½	3½
	7	5½	4	5	3½	4	8	3½	3½
	8	5½	3½	5	3½	3½	7½	3	3½
	9	5	3½	5½	3½	4	7	3	3½
	10	5	3½	5	3½	4	7	3	3½

elaboration of the statements just made. The reader will by this time have recognized that we are trying to do two things at once, first, to correlate the rather scanty venous pressure data previously available with the problem of diastolic tone and second, to do the same with our own data. We have attempted this correlation by assuming first that one theory of the behavior of the stroke volume is correct and next that the other theory of stroke volume behavior is correct; the consequences of each assumption in turn were then considered in regard to their bearing

on the question of diastolic tone. Such assumptions do not commit one as to which of the theories of the behavior of the stroke volume is correct. We do not propose to attempt to answer the question as to which of these theories is correct. We are concerned merely with the bearing of our venous pressure findings on the question of diastolic tone, regardless of which theory of the behavior of the stroke volume is correct and the only way to do this is to consider the consequences of assuming in turn that each theory is correct. We have pointed out that in the light of previously recorded venous pressure data (7), (8) the advocates of a varying stroke volume during and after exercise should have supported the idea of diastolic tone, although as a matter of fact they have been its strongest opponents, while the advocates of a constant stroke volume have really had no conclusive evidence one way or the other. On the other hand, in the light of venous pressure data reported above in this paper neither theory of the behavior of the stroke volume needs to assume a diastolic tone in order to explain the results. We may say, then, at this stage, that, assuming our finding of a rapid fall to normal of venous pressure after exercise to be correct, as opposed to the previous reports of a persistence for several minutes after exercise of the elevation of venous pressure, there is no evidence of the existence of diastolic heart tone regardless of which view we accept as to the behavior of the stroke volume.

The exercise employed in all of the experiments reported above was light, the average of external work, neglecting friction, being about 140 kgm. M. per minute. It seemed possible that the departure of our venous pressure findings from those previously reported might have been due to the fact that our work was lighter. Another series of experiments was therefore carried out in which the work was much heavier. The bicycle ergometer was used, the exercise period being fifteen minutes. The brake was set so that the fifteen minute exercise period produced a moderate degree of fatigue. The severity of the exercise was presumably about the same as in the experiments of Hooker and of Schneider, Cheley and Cisco, who also used the bicycle ergometer. In this second series of experiments the subject sat on the bicycle for at least five minutes before any readings were made. Four control determinations of venous pressure were then made at one minute intervals with the subject at rest. Only venous pressure determinations were made, the nature of the exercise rendering arterial pressure readings impossible. At a signal from the experimenter the subject started to pedal and venous pressure readings were taken at one minute intervals during the exercise period. At the end of fifteen minutes the subject stopped pedaling and rested quietly for another fifteen minutes during which venous pressure readings were continued at one minute intervals. During the first two minutes after exercise readings were made at intervals of thirty seconds.

XUM

The results are seen in table 3. In one experiment venous pressure readings were made at fifteen second intervals during a three minute

TABLE 3

	TIME	SUBJECT						
		M	D_1	D_2	H	He	T	F
	minutes							
Rest control...	1	4½	8	12½	9	16½	8½	8
	2	4½	8	12½	9½	16	8	7½
	3	4½	8	13	9½	16½	9	8
	4	4	8	12½	9½	16½	9	8
Work.....	1	6½	12½	13	15½	16½	12½	15
	2	7	13	10½	17½	16½	11	14
	3	7	11	11½	20	16½	11½	14
	4	8	13½	11½	15	15½	11½	14
	5	7½	14½	11	15	15½	11	14
	6	7	15½	11	14½	15	10½	14½
	7	7	16	11	14½	15½	10½	14½
	8	6½	15½	11	14	15½	10½	14½
	9	6½	15	10½	15	15	11	15
	10	6	15	11	15½	15	11½	14½
	11	6½	14½	11	15	14½	13	14½
	12	6½	13½	10½	14½	13½	13½	13
	13	6	13½	10½	12	13	12½	12
	14	5½	12½	10½	9½	12½	11	11
	15	5½	12	11	9	12½	10½	11
After work	½	4	8½	11½	7	11	11½	8½
	1	3½	8	12	6½	11	10½	7
	1½	3½	7½	12½	7	11½	11	6½
	2	3½	8½	9	7	12½	11	6½
	3	4	8	9	7	12½	9½	6½
	4	4	8	10	7	12½	9½	6½
	5	4	9	11	7	12½	9½	6½
	6	4	8½	11½	6½	13	10½	6½
	7	4	8½	11½	7	13	11	6½
	8	4	8	11	7	13	13	7
	9	4½	8	11	7½	13	11	7
	10	4	8	11	7½	13	12½	6½
	11	4	8	12	7½	13	11	6
	12	4	7½	12	7½	13	12	6
	13	4	8	12½	8	13	8	6½
	14	4	8	11½	8½	13½	7½	6½
	15	4	8	11½	8½	13½	8	7

period of maximum work and followed at fifteen second intervals for three minutes after work. The results are seen in table 4. The figures represent venous pressures in centimeters of water.

We had rather expected to find that with the more severe exercise the elevation of venous pressure would persist for some time after exercise (7), (8). Greatly to our surprise, however, we found that while in most cases the elevation of venous pressure during the first few minutes of exercise was more pronounced than that seen with the lighter exercise employed in the first series of experiments, the venous pressure began to fall during the actual period of exercise and in some cases had fallen below the resting level before the exercise was stopped. There was, with one exception (T), a further fall immediately on cessation of exercise so that in every case (except T) the first reading obtained after exercise stopped was lower than the control readings. In two cases, He and D_2, the venous pressure was never elevated during exercise, being at all times down to or below the control level. These subjects (except E) had all served

TABLE 4

TIME	REST	WORK	AFTER WORK
minutes			
¼	11	13	10
½		14	9½
¾		14½	9
1	11	13½	9½
1¼		13	10
1½		13	10
1¾		12	10½
2	10½	11½	10½
2¼		12	11
2½		12½	11
2¾		12	11
3	11	12	11

as subjects in the first series of experiments and may be identified by their initials. Table 4, giving the results obtained in the period covering an intense exercise of three minutes' duration and the three minutes following this exercise, during which venous pressure readings were made at fifteen second intervals, shows that the fall of venous pressure on cessation of exercise is very rapid.

We may now consider the bearing of these results on the question of diastolic tone. As before, let us first accept the view that the stroke volume increases during exercise and falls rapidly after exercise. On this view we have in these experiments in the first part of the exercise period an increased filling pressure accompanied by an increased extent of filling. So far there is no need to assume a diastolic tone since this result would be expected if the ventricle merely fills passively. As the exercise progresses, however, the venous pressure begins to fall and has

sometimes reached even a subcontrol level before the exercise stops. There is no reason to assume a concomitant decrease in the stroke volume; all the evidence for an increased stroke volume during exercise points to the conclusion that this increase of stroke volume is maintained throughout the exercise. We have, then, in the latter part of the exercise period the phenomenon of a filling pressure as low as or lower than that obtaining during rest while at the same time the stroke volume is much greater than that during rest. This necessitates the assumption that at this time the diastolic tone of the heart (using "tone" in the sense of resistance to deformation) is decreased, thereby permitting an increase in the rate of filling. On cessation of exercise, with a further fall in venous pressure, the diastolic size returns rapidly to normal. Since this post-exercise fall is in some cases slight (the level of venous pressure having already come down to or below the control level while the exercise is still in progress) we may regard the rapid decrease in diastolic volume after exercise as being an indication of a resumption of tone.

We may now consider the consequences of accepting the view that the stroke volume is constant. During the first part of the exercise period the filling pressure is increased with of course no increase in the extent of filling. Whether or not the assumption of an increase in tone is necessary depends upon whether or not we are willing to accept the view that the rate of filling is independent of the filling pressure and these experiments throw no light on this question. The same may be said of the later fall in venous pressure during exercise and the further fall after exercise. The mere absence of fluctuations in the extent of filling does not necessarily indicate fluctuations of tone. A constancy of stroke volume is compatible with a passively dilating heart if one only assumes that the venous pressure at rest can produce an adequate filling, and this assumption is necessarily made by the advocates of a constant stroke volume. To state the conclusions briefly, if one accepts the view of a constant stroke volume he cannot draw any conclusions from venous pressure data as to the presence or absence of tone until more direct evidence on the question of the rate of diastolic filling in the intact animal has been presented.

A further conclusion to be drawn from the observation that the venous pressure may return to or below its resting level during exercise and while the increase in heart rate is still being maintained is that the increase in venous pressure cannot be so important a factor in bringing about the increased heart rate under physiological conditions as Bainbridge (34) and Lassa and Miyazaki (35) have concluded. Bainbridge has not been willing to say that the effective stimulus to this reflex acceleration is the increased venous pressure *per se* but says (36) that it is "more probably a sufficient increase in the diastolic volume of the heart to

set up a slight diastolic tension in its muscular fibres." We might reconcile this second view with our own findings. As the heart loses tone its increase in diastolic volume and "tension" (slight stretching) can be maintained even after the venous pressure has returned to normal; the increased heart rate would thus be maintained.

In this paper the word "tone" has been used to designate the degree of resistance to deformation. This is the sense in which the word is applied to other hollow muscular organs as the bladder, stomach or intestine. Starling (37) employs the term "as synonymous with physiological condition or fitness of the muscle fibre, and its measure is the energy set free per unit length of muscle fibre at each contraction of the heart." Meek and Eyster (27) apparently use the term in Starling's sense. There is of course no objection to employing the term in either sense so long as one recognizes clearly in which sense it is being employed. Failure to realize just what the term means has been the souce of considerable confusion.

The writer's personal opinion on the question of the behavior of the stroke volume is that the preponderance of evidence is on the side of an increase in stroke volume during exercise. On this view one of the factors determining the efficiency of a heart is its capacity to fill under a moderate filling pressure. A loss of tone, i.e., a decreased resistance to filling, would indicate a more efficient action. Our own interpretation of the results of these two series of experiments follows. In the first series, with very light exercise, the elevation of venous pressure was maintained throughout the exercise period. The exercise period in these experiments, however, was only ten minutes. In order to determine whether or not the venous pressure would return toward its control level, as it did in the fifteen-minute periods of rather heavy exercise, if the exercise period with this lighter form of exercise were lengthened to fifteen minutes, we performed a series of experiments on seven subjects, using the same type of exercise as in the first series but lengthening the exercise period to fifteen minutes. In other words, we gave the venous pressure as much time in which to come down as we did in the bicycle experiments. The results are in table 5. The figures represent venous pressures in centimeters of water.

We see that in this series, using light exercise (about 140 kilogram meters per minute) the rise of venous pressure is not so extensive as that observed on the more severe bicycle ergometer exercise. Furthermore, it is less than that seen in the first series where exercise of the same type and severity was employed. The fact that the rise of venous pressure in this last series averages less than in the first series may be due to fact that the first series was carried out during cold weather when the room temperature was about 20°C., while the last series was carried out during warm

weather with the temperature about 27°C. We know of no other difference in the experimental conditions of the two series. The failure of the rise

TABLE 5

	TIME	SUBJECT						
		D	He	H	Z	M	Dv	T
	minutes							
Rest control...	1	4½	8½	5	5	6	6	8½
	2	4½	9	5	5½	6½	6½	8
	3	4½	9	5	5½	6½	6½	8
	4	5	9	5	5½	6½	6½	8½
Work.....	1	5½	9½	6	6½	6	6½	9
	2	6	9½	6	8	6½	6½	9
	3	6	10	6½	8½	7	6	9½
	4	6	9½	6	8½	7	6	10
	5	6½	10	6½	9	7	6	10
	6	6½	10	6½	8½	7	6	10½
	7	6½	10	6½	8½	7	6	10½
	8	6½	9½	7	8	7	6	10½
	9	6	9½	6½	8½	7	6	9½
	10	6½	8½	6½	8½	6½	5½	10
	11	6½	8½	6	8	7	6	9½
	12	7	9	6	8	6½	6	9½
	13	6½	9½	6½	8½	6½	6	9
	14	6½	9	6	8	7	6	9
	15	6½	9½	6½	8½	7	6	9½
After work	½	4½	8	4	6	6	5	8½
	1	5	8	4	6	5½	5	8½
	1½	5½	8	4½	5½	6	5½	8
	2	4½	8½	4	6	6	5½	8½
	3	5	9	4½	5½	6½	6	8½
	4	4½	9	4½	5	7	6	8½
	5	4½	9	5	5	6	6	8½
	6	4½	8½	5	5½	5½	6	8
	7	5	9	4½	5½	5½	6	6½
	8	4½	8½	4½	5½	5½	6	7
	9	4½	8½	4½	6	6	6	6½
	10	4½	8½	5	5½	6	6	7
	11	5½	9	4½	5½	5½	6	7
	12	5½	9	4½	5	6	6	6½
	13	4½	9	5	5½	6	6	6½
	14	5	8½	5	5	6	6	7
	15	4½	9	5	5½	6	6	6½

of venous pressure in the last series might then be explained on the basis that the greater heating during exercise effected a greater decrease in

diastolic heart tone, thus lowering the resistance offered to the inflow of blood.

We are now ready to state our interpretation of these findings. We believe that at the onset of exercise the venous return is increased by the various factors generally recognized. The heart in the face of this increased venous return at first maintains its resting tone or resistance to filling. The result is an increase of venous pressure which results in a more complete filling. There is little or no loss of tone, neither is there an increase, the immediate increase in stroke volume being due entirely to the increased filling pressure. If the exercise is very light the resting tone is maintained throughout the exercise or only slightly decreased, with the result that, since the venous return remains high, the venous pressure remains high. With heavier exercise, however, the heart after a few minutes begins to lose tone, i.e., offers less resistance to the inflow of blood, with a consequent fall in venous pressure. With a higher temperature of environment lighter exercise may produce the same effect which is seen on heavier exercise at a lower temperature. This means that the increased extent of filling can now be effected with a filling pressure lower than that required at the onset of exercise, before the heart's tone had decreased. When the exercise stops the heart resumes its resting tone and returns rapidly to its resting size and stroke volume. It would appear, then, that the lighter the exercise the less the resulting decrease in diastolic heart tone. The suggestion is also made that a higher temperature may accentuate the loss of tone.

It is interesting to note that the fall of venous pressure during heavy exercise, taking place usually about ten minutes after onset of exercise, which fall we have just considered an index of an increased cardiac efficiency, is usually accompanied by the onset of sweating. After this association had been noted the subjects were asked at the beginning of the experiment to tell when they noticed the development of "second wind" (38) but in no case could the subjective sensation of second wind be definitely correlated with the fall of venous pressure. It seems quite reasonable, however, that the increased capacity to do work which develops after work has been in progress for a few minutes has at least as one of its causative factors the increased cardiac efficiency signified by the heart's ability to fill under a decreased filling pressure. It is probable that this decrease in tone has a chemical rather than a nervous basis but with present data we could only speculate as to its exact nature. The variations in tone may well depend upon variations in CO_2 tension or possibly pH of the plasma.

A word may be said here about the case of T. The failure of his venous pressure to fall during the latter part of his heavy exercise period may mean that his heart is less efficient in that it does not lose tone, i.e., acquire

XUM

the capacity of dilating against a lower filling pressure. This subject, however, shows no evidence of heart disease although he is considerably under weight. Any assertion as to the possibility of drawing conclusions about a heart's efficiency from the venous pressure response to exercise would be premature here.

A word may be added here in regard to the *a priori* aspects of the subject. Henderson's view is that diastolic tone increases during exercise, thereby affording a greater resistance to the venous inflow at the very time when one might reasonably expect this inflow to be facilitated. This view was of course formulated in the absence of any data on a fall of venous pressure taking place in the latter phase of an exercise period. On purely *a priori* grounds a decrease in the resistance afforded to the inflow of blood to the heart during exercise would seem more conservative of the organism than would an increase.

SUMMARY

Previous workers (7), (8) have reported an increased venous pressure persisting for several minutes after exercise. Those physiologists who hold the view that the stroke volume of the heart increases during and falls rapidly after exercise have accepted these findings without question and have at the same time denied diastolic heart tone. The inconsistency of these two points of view is pointed out.

The findings reported in this paper are an elevation of venous pressure maintained more or less completely during light exercise up to fifteen minutes and falling immediately to or below the control level on cessation of exercise. With heavy exercise the elevation of venous pressure in the early part of the exercise period is usually more pronounced than that seen on light exercise, but after several minutes the venous pressure begins to fall and has usually returned to or below the control level before exercise stops. On cessation of exercise there is usually a slight further drop. In general it may be said that the tendency to return toward normal after about eight to ten minutes is more pronounced the heavier the exercise, at least up to the heaviest employed by us.

A higher temperature apparently favors the fall of venous pressure which occurs during exercise.

The effective stimulus to the reflex acceleration of heart rate during exercise is more probably the result of an increased diastolic volume than of an increased venous pressure *per se*. The acceleration may be maintained after the venous pressure has returned during exercise to its resting level.

The interpretation of these findings as bearing on the question of diastolic tone is discussed, first on the view that the stroke volume of the

heart varies on exercise and second, on the view that the stroke volume is constant.

The writer's personal interpretation of the findings is that the heart muscle during rest possesses tone and that this tone suffers little or no change on light exercise and in the early part of heavy exercise. After several minutes of heavy exercise the tone decreases, permitting a more efficient filling; a high temperature of the environment facilitates the loss of tone. On cessation of exercise the heart rapidly takes on its resting tone.

BIBLIOGRAPHY

(1) GASKELL: Journ. Physiol., 1880, iii, 53.
(2) HENDERSON: This Journal, 1906, xvi, 325.
(3) HENDERSON: This Journal, 1909, xxiii, 345.
(4) HENDERSON: Physiol. Reviews, 1923, iii, 165.
(5) YAMADA: Mitteil. d. med. Fakultat. d. kais. Univ. zu Tokio, 1916, xvi, 527.
(6) BAINBRIDGE: The physiology of muscular exercise. 2nd ed., London, 1923, 52.
(7) HOOKER: This Journal, 1911, xxviii, 235.
(8) SCHNEIDER, CHELEY AND CISCO: This Journal, 1918, xl, 380.
(9) KROGH AND LINDHARD: Skand. Arch. f. Physiol., 1912, xxvii, 100.
(10) KROGH AND LINDHARD: Journ. Physiol., 1913, xlvii, 112.
(11) LINDHARD: Pflüger's Arch., 1915, clxi, 233.
(12) LINDHARD: Journ. Physiol., 1922, lvii, 17.
(13) BOOTHBY: This Journal, 1915, xxxvii, 383.
(14) DOUGLAS AND HALDANE: Journ. Physiol. 1922, lvi, 69.
(15) BINGEL: Münch. med. Wochenschr., 1907, liv, 57.
(16) DE LA CAMP: Zeitschr. f. klin. Med., 1903, li, 1.
(17) LENNHOFF UND LEVY-DORN: Deutsch med. Wochenschr., 1905, xxxi, 869.
(18) MENDEL UND SELIG: Med. Klinik, 1907, iii, 135.
(19) HOFFMANN: Verhandlungen d. Kongress f. innere Med., 1902, xx, 307.
(20) KIENBOCK, SELIG UND BECK: Münch. med. Wochenschr., 1907, liv, 1427.
(21) BAINBRIDGE: Loc. cit., 65.
(22) NICOLAI UND ZUNTZ: Berl. klin. Wochenschr., 1914, li, 821.
(23) MORITZ: Münch. med. Wochenschr., 1908, lv, 713.
(24) HENDERSON: Physiol. Reviews, 1923, iii, 202.
(25) DIETLEN: Ergebn. d. Physiol., 1910, x, 598.
(26) BRUNS: Münch. med. Wochenschr., 1921, xviii, 907.
(27) MEEK AND EYSTER: This Journal, 1923, lxiii, 400.
(28) STARLING: Linacre Lecture on the law of the heart, 1915, 6.
(29) BAINBRIDGE: Loc. cit., 69.
(30) KROGH: Skand. Arch. f. Physiol., 1912, xxvii, 131.
(31) HENDERSON: This Journal, 1906, xvi, 346.
(32) KROGH: Skand. Arch. f. Physiol., 1912, xxvii, 132.
(33) WHITE: This Journal, 1924, lxix, 10.
(34) BAINBRIDGE: Journ. Physiol., 1915, l, 65.
(35) LASSA AND MIYAZAKI: Journ. Physiol., 1920, liv, 203.
(36) BAINBRIDGE: Loc. cit., 78.
(37) STARLING: Loc. cit., 26.
(38) PEMBREY AND COOKE: Journ. Physiol., 1908, xxxvii, lxvii.

THE ACTION OF PREPARATIONS OF THE ENDOCRINE GLANDS UPON THE WORK DONE BY SKELETAL MUSCLE

NATHAN B. EDDY

From the Department of Physiology and Pharmacology, University of Alberta, Edmonton, Canada

Received for publication April 22, 1924

In December, 1921, experiments were undertaken in this laboratory upon the general problem of the relation of the internal secretions to the activity of skeletal muscle. About a year later the report of Yoshimoto's work appeared (1) under the title, "The action of extracts of the endocrine glands upon motor nerve and skeletal muscle." He investigated particularly the excitability of isolated nerve and muscle immersed in solutions of endocrine extracts, whereas we chose for our starting point the determination of the extent to which the working power of muscle is affected when it is perfused with a solution of a gland extract or an internal secretion. So different were these methods of attacking the general problem we had in mind that the work has been continued and the results to date are reported herewith.

Yoshimoto gives a comprehensive review of the scanty literature so that reference to it will be confined to comments, a little later, upon the points of agreement or disagreement.

Our method of procedure was adapted from one employed by Miss Scarborough (2) in a pharmacological investigation. It consisted essentially in recording the exhaustion curve of each gastrocnemius muscle of a frog, uniformly loaded and uniformly stimulated,—the one during perfusion of the muscle through the general circulation with Ringer's solution only, the other during perfusion in like manner with Ringer's solution plus a gland extract or internal secretion.

While it is true, as Miss Scarborough (2) points out, that the two gastrocnemii of the same frog may vary widely in their excitability and the character of the exhaustion curve, this is only infrequently the case. In attempting to determine the effect of any agent upon the working power of a muscle we know of no better standard of comparison than the other muscle of the pair working under the same conditions except for the agent being investigated. If the initial response of each muscle under as nearly as possible identical conditions of stimulation, mechanical arrangement and

perfusion is the same, and if the differences in the exhaustion curve of one of these muscles are uniformly of one kind in a series of trials, all conditions remaining the same except the character of the perfusion fluid, we believe we are justified in assuming that such differences in the exhaustion curve are the effect of the modification of the perfusion fluid. Upon this assumption we have proceeded in the conduct of this investigation, ruling out of consideration any experiment in which the initial response of the companion muscles was not the same within very narrow limits.

Medium sized healthy winter frogs (Rana pipiens) from Indiana and Illinois sources were used. Both brain and spinal cord were completely pithed. A side-arm cannula was inserted into the bulbus arteriosus through an incision into the ventricle. This cannula was connected with a series of funnels filled with Ringer's solution, elevated to give a practically constant pressure of 30 to 40 cm. of water. The pressure varied with different sized frogs, not with the same frog. The side-arm of the cannula permitted ready exclusion of air and facilitated accuracy in determining the time at which the experimental solution began to enter the frog's vessels. The perfusion was carried out at room temperature. The composition of the Ringer's solution was sodium chloride 0.65 gram, potassium chloride 0.03 gram, calcium chloride 0.026 gram, distilled water to 100 cc.

To determine whether the exhaustion curve would vary as the quantity of the fluid perfused the latter was measured during each portion of an experiment. However, the measurement was not made minute by minute, so that conclusions cannot necessarily be drawn as to the immediate effects of the experimental solution upon the caliber of the blood vessels. Nevertheless such effects were noted incidentally in some cases.

It was readily shown, in confirmation of Miss Scarborough (2), that the perfusion fluid penetrated the gastrocnemius muscle quickly and efficiently. After passing through the frog's circulatory system the fluid escaped through the incised ventricle, which continued to beat under its influence.

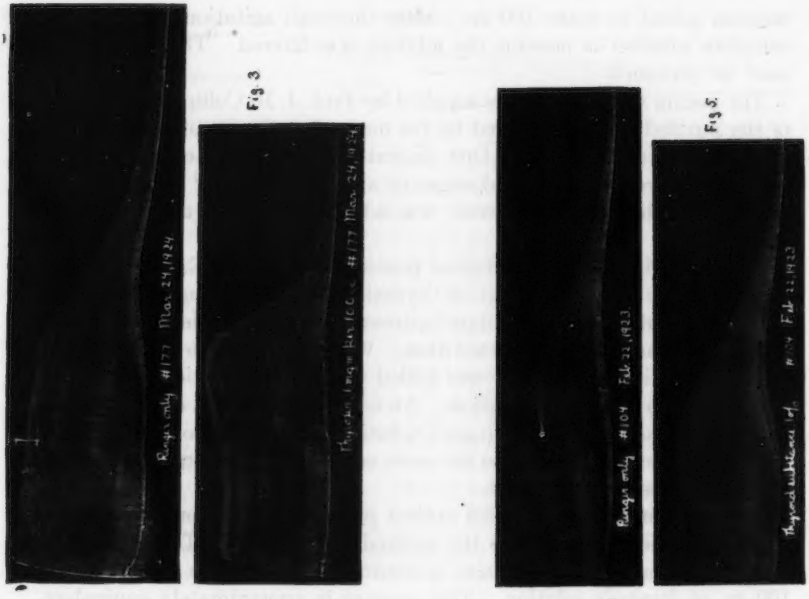
Through a small incision in the skin the tendon of each gastrocnemius was cut below the sesamoid bone. The muscle was not otherwise disturbed, especial care being taken to avoid traction on it before the beginning of the experiment. A piece of fine copper wire was fastened securely to the tendon and by means of it connection was made with a crank myograph arranged to record on a very slow drum. Each leg was fixed by a pin through the knee-joint and supported in the horizontal position, since this facilitates the perfusion. The body of the frog was supported vertically. Each muscle, only while its exhaustion curve was being recorded, was free-weighted with 20 grams. The points of attachment of weight and muscle were equidistant from the pivot of the myograph. The muscle lever magnified the shortening of the muscle twelve times. Stimuli

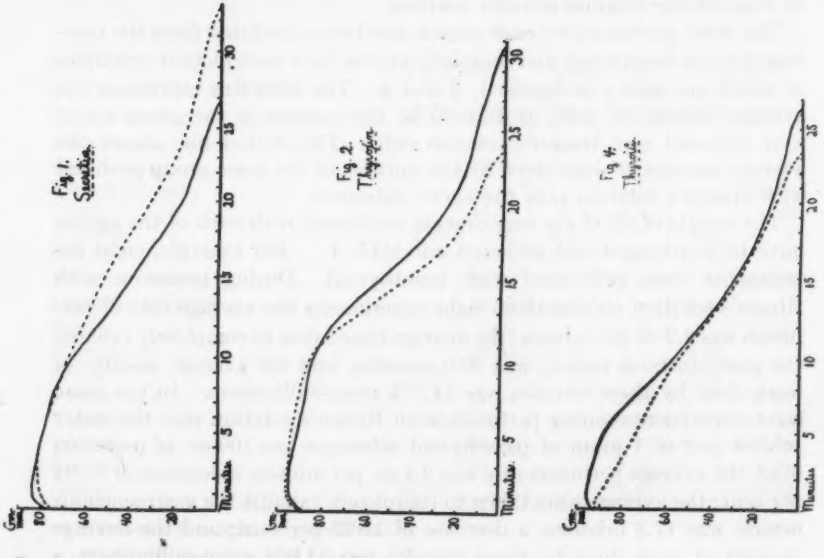
were single maximal break shocks (make shocks short-circuited) from the secondary circuit of a du Bois Reymond induction coil and were uniform for both muscles of a pair. The current was led into the upper end of the muscle through the pin in the knee-joint and into the other end of the muscle by way of the wire attaching the tendon to the myograph, thus ensuring through and through stimulation, while at the same time avoiding all injury to the muscle. No other path for the current was possible. The stimuli were sent in at intervals of five seconds.

Perfusion of the preparation with Ringer's solution only having been established and continued until the fluid ran clear (practically blood-free) from the ventricle, the strength of the maximal break shock and the height of response which it would produce were determined for one gastrocnemius muscle. The drum was started and the contractions of this muscle to this strength shock recorded to the point of complete exhaustion. The time which this required and the total fluid perfused during this time were noted. Then the other muscle was similarly attached to the myograph and its response to the same strength shock determined. If this was like the initial response of the first muscle, perfusion was begun with Ringer's solution plus the specific agent under investigation. At the end of a minute the record of the second exhaustion curve was started. Again fatigue time and total amount of fluid perfused were noted. No attempt was made to determine to what extent the muscles would recover under continued perfusion with Ringer's solution only.

We have carried out experiments using the following agents: Substance of mammary, pineal, parathyroid, thyroid, thymus and suprarenal glands, pancreas, spleen, ovary, testis, corpus luteum, anterior and posterior lobes of the pituitary body, insulin, thyroxin and secretin. Of these extract of suprarenal (or adrenalin) has received most detailed attention by other investigators, the results of which have been reviewed recently by Gruber (3), (4). It has been included in this series in order to compare the results of our method with the work of others. It is frequently stated with truth that preparations of the various glands do not necessarily contain the active principle of the gland, at least in active form. On this account we have included a preparation of the whole pancreas and insulin, a thyroid extract and thyroxin, and suprarenal extract rather than adrenalin, believing that, if the effects of gland substance and isolated active principle should prove comparable in these three cases, there would be some reasonable justification for attributing effects of the other gland substances to their content of specific active ingredient.

The gland substances employed were the dried powders prepared by Armour & Company. One gram of the powder was rubbed up in a mortar with a few cubic centrimeters of Ringer's solution, then enough Ringer's





See text, page 436, for explanation of figures.

solution added to make 100 cc. After thorough agitation to obtain as complete solution as possible the mixture was filtered. The filtrate was used for perfusion.

The insulin was very kindly supplied by Prof. J. B. Collip and was part of the purified insulin prepared by his method at the Connaught laboratories, Toronto University. One clinical unit (one-third of the amount necessary to reduce the blood sugar of a rabbit weighing 2 kilos to the point at which convulsions occur) was added to each 100 cc. of Ringer's solution.

The thyroxin was the crystalline powder prepared by E. R. Squibb & Sons. To the desired amount of thyroxin (from 3 to 5 mgm.) one drop of a 10 per cent solution of sodium hydroxide was added followed by about a cubic centimeter of Ringer's solution. When the thyroxin had dissolved completely Ringer's solution was added to give a proportion of 1 mgm. of thyroxin to 100 cc. of solution. An equivalent amount of sodium hydroxide was added to the Ringer's solution for the control perfusion in this series of experiments. So far as we could detect the trace of sodium hydroxide was without effect.

The secretin was a dried acid extract prepared as previously described (5) and purified according to the method of Stepp (6). Ten milligrams of the final powder, which gave a neutral solution, were added to each 100 cc. of Ringer's solution. This amount is approximately equivalent to 2 cc. of our original secretin solution.

The work performed by each muscle has been calculated from the tracings in every experiment and composite curves have been plotted, examples of which are shown in figures 1, 2 and 4. The solid line represents the average amount of work performed by the muscles in the group which were perfused with Ringer's solution only. The dotted line shows the average amount of work done by the muscles of the same group perfused with Ringer's solution plus the agent indicated.

The results of all of the experiments performed with each of the agents have been averaged and gathered into table 1. For example, eight experiments were performed with parathyroid. During perfusion with Ringer's solution only in these eight experiments the average rate of perfusion was 4.2 cc. per minute; the average time taken to completely exhaust the gastrocnemius muscle was 20.9 minutes, and the average amount of work done by these muscles was 14,572 gram-millimeters. In the same eight experiments during perfusion with Ringer's solution plus the water soluble part of 1 gram of parathyroid substance per 100 cc. of perfusion fluid, the average perfusion rate was 3.4 cc. per minute, a decrease of 19.04 per cent; the average time taken to completely exhaust the gastrocnemius muscle was 17.3 minutes, a decrease of 17.22 per cent; and the average amount of work done by these muscles was 11,994 gram-millimeters, a

TABLE 1

SUBSTANCE EMPLOYED	NUMBER OF OBSERVATIONS	PERFUSION RATE PER MINUTE		FATIGUE TIME		WORK DONE	
		A	B	A	B	A	B
		cc.	*cc.*	*minutes*	*minutes*	*gm.-mm.*	*gm.-mm.*
Mammary	17	5.1	3.6	23.3	23.9	11,988	14,080
Ovary	11	5.3	4.6	20.0	20.0	11,368	11,302
Corpus luteum	9	4.0	2.2	20.0	16.5	16,048	13,724
Testis	10	4.5	3.1	19.5	18.6	13,430	13,646
Thymus	18	3.6	3.6	22.3	25.4	12,986	13,296
Pineal	10	4.2	3.1	19.5	21.7	13,500	15,468
Anterior pituitary	13	5.1	2.3	28.7	22.2	16,204	13,282
Posterior pituitary	12	4.8	2.6	29.0	29.3	16,874	17,542
Spleen	11	4.4	4.0	27.1	22.2	16,342	14,058
Suprarenal	9	5.6	2.8	21.0	22.0	13,418	14,630
Secretin	6	5.9	7.8	26.8	33.0	15,610	18,164
Parathyroid	8	4.2	3.4	20.9	17.3	14,572	11,994
Thyroxin	8	5.4	5.9	30.5	23.8	20,080	17,836
Thyroid	10	4.0	2.8	26.3	23.4	16,260	13,704
Pancreas	14	6.5	4.3	22.6	20.9	14,120	13,230
Insulin	5	4.1	5.9	21.8	19.0	13,068	11,264

A, during perfusion with Ringer's solution only.
B, during perfusion with Ringer's solution plus specific substance employed.

TABLE 2

Percentage differences

SUBSTANCE EMPLOYED	PERFUSION RATE		FATIGUE TIME		WORK DONE	
	Increase	Decrease	Increase	Decrease	Increase	Decrease
Mammary		29.41	2.57		17.45	
Ovary		13.20*	0.00	0.00		0.58
Corpus luteum		45.00*		17.50		14.48
Testis		31.11		4.61		1.60
Thymus	0.00	0.00	13.90		2.40	
Pineal		26.19	11.28		14.54	
Anterior pituitary		54.90*		21.95		18.03
Posterior pituitary		45.83	1.03		3.95	
Spleen		9.09		18.08		13.97
Suprarenal		50.00	4.76		9.03	
Secretin	32.20		23.17		16.33	
Parathyroid		19.04		17.22		17.69
Thyroxin	9.25			20.96		11.17
Thyroid		30.00*		11.78		15.75
Pancreas		33.84		7.52		6.01
Insulin	43.90			12.84		13.80

*Perfusion rate primarily increased.

decrease of 17.69 per cent. These percentage differences for each group of experiments are represented in table 2.

DISCUSSION. Extracts of the ovary and testis did not affect particularly either the total work performed by the gastrocnemius muscle of the frog or the development of fatigue. Extract of the posterior lobe of the pituitary primarily decreased the height of contraction, manifested particularly by an absence of treppe, but the total work done under its influence was slightly increased.

Extracts of the pineal, suprarenal, thymus and mammary glands and our preparation of secretin increased the work done by skeletal muscle and delayed the development of fatigue. The pineal extract affected work and fatigue time about equally. The effect of suprarenal extract was somewhat greater, and that of extract of mammary gland very much greater upon the work done than upon the development of fatigue. On the other hand, thymus extract delayed materially the complete exhaustion of the muscle without greatly increasing the total work done.

Extracts of parathyroid and thyroid glands, anterior lobe of the pituitary and corpus luteum, pancreas and spleen, thyroxin and insulin decreased the work and shortened the time required for the development of complete fatigue in the frog's gastrocnemius. Except in the case of thyroxin these two effects were about equal. With this substance the fatigue time was shortened much more (20.96 per cent) than the work was decreased (11.17 per cent), on account of an increased height of contraction in the early part of the experiment. This is particularly well shown in the accompanying tracing (fig. 3). Thyroid gland extract occasionally (three out of ten experiments) showed similar improvement in the height of contraction in the early part of the curve (fig. 5). Thyroxin stimulates metabolism, or, according to Boothby and Sandiford (7), "The function of thyroxin is to regulate the capacity of a cell to use oxygen," or again, according to Kendall (8), thyroxin catalyzes oxidation. If this catalysis were to occur at a faster rate than the contracting mechanism of skeletal muscle could transform the liberated energy into mechanical work, there would result more or less increase in the height of contraction but at the same time fuel wastage, becoming apparent in a more rapid appearance of complete exhaustion in the muscle.

In our hands both pancreatic extract, made from the whole gland, and insulin decreased the work done by muscle and hastened the appearance of fatigue, effects directly antagonistic to those of suprarenal extract.

Extracts of the thyroid gland, anterior lobe of the pituitary, pancreas, ovary and corpus luteum caused primarily an increase in the rate of perfusion but a net decrease for the whole period of observation. Extracts of parathyroid, pineal, suprarenal and mammary glands, posterior lobe of the pituitary, spleen and testis decreased the amount of fluid perfused

without apparent primary increase in the rate of flow. Thyroxin, insulin and secretin always increased the perfusion rate. Thymus extract had no effect upon the quantity of fluid perfused. A comparison of these results with the changes already noted in work and fatigue time produced by the same agents makes immediately obvious the absolute lack of parallelism between the two effects (see table 2). For example, secretin and suprarenal extract both increased the work of muscle, but the former increased the perfusion rate while the latter decreased it. Again, extracts of the pineal and parathyroid glands both decreased the rate of perfusion, but the former increased the work done and the latter decreased it. The net effects of extract of pancreas and of insulin were opposite upon the perfusion rate but identical in kind upon the muscle.

The work of other investigators in studying the effect of adrenalin upon skeletal muscle has been mentioned (3). In general they report improvement in the working power of muscle under the influence of adrenalin and antagonism between adrenalin and the development of fatigue in a muscle. Our results with an extract of suprarenal gland are in accord with these findings.

Yoshimoto (1) studied especially the excitability of nerve and muscle but he makes some comments upon variations in the height of muscular contractions (frog's sartorius) in response to direct, single, widely spaced stimuli. He obtained with extract of the posterior lobe of the pituitary a slight initial increase in the height of contraction, not apparent when extracts free from the alcohol soluble depressor substance were used. Extracts of the anterior lobe of the pituitary were without consistent effect; in some experiments the height of contraction was increased, while in others it was decreased. Extracts of brain sustance, parathyroid, thyroid and thymus glands were without demonstrable effect. Extracts of pineal and suprarenal glands, testicle and ovary decreased the height of contraction. It is worthy of note that he obtained the most effect, always depressive, with extract of suprarenal gland. Our results do not agree entirely with these. Especially is this true of the effects of pineal and suprarenal extracts. Possibly the differences are attributable to the different methods employed.

Müller (9) reported and Del Campo (10) confirmed the observation that fresh thymus extracts caused temporary disappearance from muscle of fatigue that was being induced by rhythmical stimulation. Our experiments again confirm this result.

Rogers, Coombs and Rahe (11) reported increase in the vigor of fatigued voluntary muscle, rectus abdominis of the cat, after intravenous injection of extracts of thyroid, parathyroid or adrenal glands. They employed a number of other gland extracts in like manner without effect upon the muscle. The statement was made that desiccation of the thyroid lessens

its activity. Our results are contradictory to the above except in the case of suprarenal gland. However, we have commented upon a possible mode of action of thyroxin and of thyroid in so far as it contains thyroxin.

It is admitted that the gap in our knowledge of the endocrine glands in their relation to the activity of skeletal muscle has barely been touched. Many interesting problems demand further elucidation. In connection with some of these further work is already planned.

CONCLUSIONS

1. Perfusion of the frog's gastrocnemius muscle with preparations of the endocrine glands or with internal secretions affects the work of muscle as follows:

a. Parathyroid, corpus luteum, anterior pituitary, thyroid, splenic and pancreatic substances, insulin and thyroxin decrease the work done.

b. Pineal, suprarenal, thymus and mammary substances and secretin increase the work done.

c. Ovarian, orchic and posterior pituitary substances do not affect materially the work done.

2. The effect upon work done of suprarenal substance and adrenalin is identical in kind, as is that of thyroid substance and thyroxin, and of pancreatic substance and insulin.

3. The work done during perfusion with all of the agents studied varies independently of the perfusion rate.

BIBLIOGRAPHY

(1) Yoshimoto: Quart. Journ. Exper. Physiol., 1922, xiii, 5.
(2) Scarborough: Journ. Pharm. Exper. Therap., 1921, xvii, 129.
(3) Gruber: Endocrinol., 1919, iii, 145.
(4) Gruber: This Journal, 1922, lxii, 438.
(5) Downs and Eddy: This Journal, 1917, xliii, 415.
(6) Stepp: Journ. Physiol., 1912, xliii, 441.
(7) Boothby and Sandiford: Physiol. Reviews, 1924, iv, 106.
(8) Kendall: Quoted by Boothby and Sandiford: Physiol. Reviews, 1924, iv, 106.
(9) Müller: Zeitschr. Biol., 1917, lxvii, 489.
(10) Del Campo: Zeitschr. Biol., 1918, lxviii, 285.
(11) Rogers, Coombs and Rahe: This Journal, 1918, xlv, 97.

THE PHYSIOLOGICAL MAXIMUM RATE OF THE HEART

JOSEPH TULGAN

From the Department of Physiology, Columbia University, New York

Received for publication April 11, 1924

Hooker (1), makes the statement that after bilateral vagotomy, the heart has a physiological maximum rate beyond which no further increase is possible. Stimulation of an afferent nerve fails, under these conditions, to give any further acceleration of the heart. Recently I made a similar statement (2), and the experiments reported here were begun with the idea of testing the validity of this statement by further evidence. In order to do this, I decided to use some stimulus other than that coming in over an afferent nerve. Adrenalin[1] was chosen, since by its intravenous injection a powerful stimulant would be carried to the heart in the blood stream.

There is strong presumptive evidence, at least, at hand now that cardiac acceleration above a certain autochthonous rate in an animal does not come about as the result of inhibition of the inhibitory mechanism; consequently section of the vagi does not in any way interfere with any increase in heart rate which we might afterwards obtain. Hunt (3) recorded the fact that he could never obtain an acceleration of the heart after double vagotomy as proof that cardiac acceleration must occur as the result of inhibition of these nerves, but he did not take into consideration the possibility of a physiological maximum heart rate after section of the vagi. Stewart (4) and his collaborators noted that the usual changes in heart rate during cerebral anemia were never so prominent after division of the vagi. This fact is now being studied in greater detail by Dr. Helen C. Coombs.[2]

The injection of adrenalin under ordinary circumstances results in a rise of blood pressure and a markedly accelerated heart, but sometimes the heart may become inhibited reflexly through the vagus mechanism if the vagi are intact. If the vagi are intact, the heart is not beating at its physiological maximum rate, if we assume that the maximum rate is reached only after double vagotomy, and consequently we would expect the possibility of an increase in rate when the vagi are intact. Stimulation of many sensory nerves gives the same results as adrenalin when the vagi are intact. The object of these experiments was to find out if there

[1] Suggested by Prof. F. H. Pike.

[2] Personal communication.

is any further increase in the rate of the heart on injection of adrenalin after the vagi are sectioned. If there is, then we are not justified in concluding that the heart has reached its physiological maximum rate after double vagotomy. On the other hand, if there is no marked increase in the heart rate, we are justified in concluding that the heart has reached its physiological maximum rate, beyond which it can not be any further increased after bilateral vagotomy.

We are justified in using adrenalin for these experiments because it is known that adrenalin acts directly on the heart. According to Stewart (5), the heart is directly stimulated by adrenalin. Levy (6) and Gasser and Meek (7) have also shown that a denervated heart will respond by a quickened beat to extremely small concentrations of adrenalin injected intravenously. The increase in rate may be as much as 28 beats per minute. Elliot (8) has shown that the action of adrenalin on any part of the body is practically identical with that of excitation of the sympathetic nerve supply to that part.

The action of adrenalin on the isolated heart is best shown on the perfused heart or in the heart-lung preparation. On adding this substance to the blood passing through such a preparation an effect is at once produced. This action is, like that of the sympathetic nerves, accelerator and augmentor. Through its influence, the rhythm of the heart is markedly increased in the dog to about 240 per minute (9).

Cannon (10) has employed the completely denervated heart as an indicator of adrenal secretion. Since this organ is no longer under the control of nervous impulses, it is acted upon only by agencies acting through the circulation. It is highly sensitive to circulating adrenalin. Afferent stimulation causes the heart, disconnected from the central nervous system, to increase to a marked degree its rate of beat. In the published reports the increments ranged from 24 to 52 beats per minute. After the adrenals were removed, there was no increase of rate or only a slight increase.

In another publication (11) the same author points out that the striking cardiac acceleration which follows afferent stimulation when the adrenals are the only responsive abdominal viscera, and the absence of this reflex acceleration when thereafter the adrenals are removed, can be explained only by the action of secreted adrenalin reflexly increased and carried to the heart in the circulation.

He further proves in the same paper that the denervated heart in the "reduced" animal is a means of assaying adrenalin since repeated equal doses injected intravenously at a uniform rate repeatedly produced in a given animal the same or nearly the same maximal increases of heart beat and if the injections differed in rate, the increases likewise differed in degree.

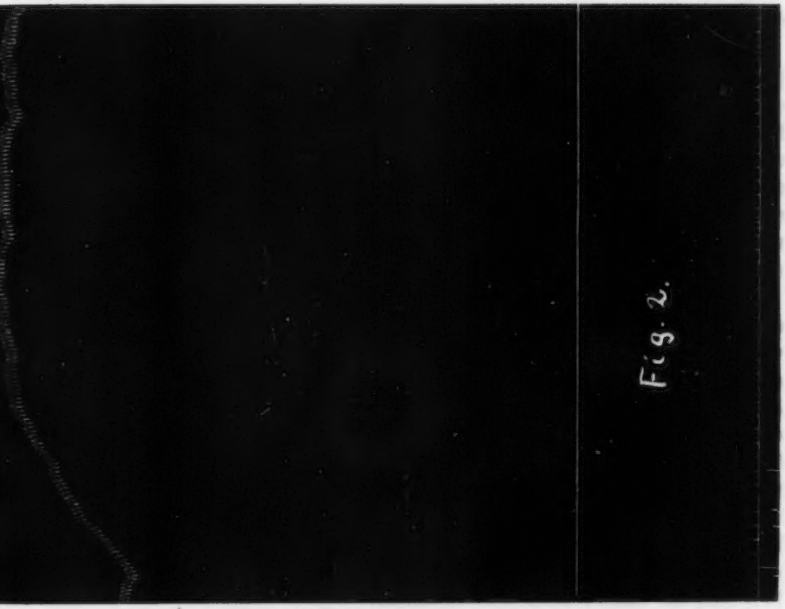


Fig. 1. Heart rate and blood pressure after bilateral vagotomy.
Fig. 2. Heart rate and blood pressure after double vagotomy followed by injection of adrenalin.

From these experimental facts it would seem that we are justified in using adrenalin in experiments in which we wish to determine whether any further acceleration of the heart rate is possible after bilateral vagotomy.

Experimental procedure. All the experiments were done on cats. Blood pressure and heart rate were recorded by means of a mercury manometer connected to the left carotid artery. Tracings were made on a Hürthle kymograph. The time was recorded in seconds by means of a Jacquet chronograph. A signal magnet served as the base line for blood pressure and also indicated the time of injection of adrenalin or of any other experimental procedure. Adrenalin was injected by means of a hypodermic needle which was plunged into the femoral vein. The heart rate was later computed in 10-second intervals.

Effect of injection of adrenalin on heart rate after double vagotomy. After a normal tracing of blood pressure and heart rate was taken, both vagi were sectioned. The blood pressure and heart rate increased, and remained at the higher level. A small amount of adrenalin was then injected into the femoral vein. Immediately the pressure rose, in some cases to double the original pressure. On computing the heart rate, however, it was found that there was no change in the rate after the injection of adrenalin from what it was after double vagotomy.

In a previous publication (loc. cit.) I have also shown that there is no increase in the rate of the heart on stimulation of a sensory nerve after double vagotomy. Further experiments of this nature were also done here and the same results were obtained.

It would seem from the results obtained here that Hooker's statement in regard to the physiological maximum rate of the heart is correct and his view is further confirmed by these observations.

	BLOOD PRESSURE	HEART RATE IN 10 SECOND PERIODS
After double vagotomy	140	35
After injection of adrenalin	180	35

SUMMARY

Twenty experiments were done on cats to determine if the heart rate could be further increased after section of the vagi, by injection of adrenalin. A tracing of blood pressure and heart rate after double vagotomy and before injection of adrenalin, and another tracing of the blood pressure and heart rate after adrenalin was injected, are given. These two tracings are representative of a great many which were obtained.

The results obtained seem to show that the heart beats at its physiological maximum after double vagotomy and that no further increase in rate

can be obtained either by injection of adrenalin or by the stimulation of a sensory nerve. The recognition of this fact would help to clear up much of the confusion in the literature regarding cardiac acceleration.

CONCLUSION

After both vagi have been sectioned, the rate of the heart is at its physiological maximum and it can not be increased any further by stimulation of a sensory nerve or by the injection of adrenalin. Hooker's statement is further confirmed by these experiments.

BIBLIOGRAPHY

(1) HOOKER: This Journal, 1907, xix, 417.
(2) TULGAN: Ibid., 1924, lxviii, 31.
(3) HUNT: Ibid., 1899, ii, 395.
(4) STEWART ET AL: Journ. Exper. Med., 1906, iii, 289.
(5) STEWART: A manual of physiology, 8th ed., 655.
(6) LEVY: Heart 1913, iv, 342.
(7) GASSER AND MEEK: This Journal , 1914, xxxiv, 63.
(8) ELLIOT: Journ. Physiol., xxxii, 401.
(9) STARLING: Human physiology, 3rd ed., 1020.
(10) CANNON: This Journal, 1919, l, 399.
(11) CANNON: This Journal, 1921-22, lviii, 308.

THE EFFECT OF ADRENALIN UPON THE PATELLAR TENDON REFLEX

W. W. TUTTLE

From the Laboratories of Physiology, Ohio State University

Received for publication April 14, 1924

That adrenalin has a marked effect upon mammalian skeletal muscle has been reported by Cannon and Nice (1), Gruber (2) and Hoskins and Durrant (3). Gruber has studied this effect most extensively and concludes that adrenalin has a specific effect upon fatigued muscle other than that due to circulatory changes.

That skeletal muscle receives sympathetic fibers has been claimed by Perroncito (4) and Boeke (5). This view is supported by DeBoer (6) and Langelaan (7), (8) who conclude that muscle tonus is maintained through sympathetic fibers. Since adrenalin is a selective sympathetic stimulant this conception furnishes a mechanism whereby adrenalin presumably could act directly upon the muscle through its sympathetic innervation. Adrian (9) points out, however, that although the sympathetic system is undoubtedly connected with skeletal muscle "there is an important body of evidence which is directly opposed to the view that the sympathetic has anything to do with the development or the maintenance of tone."

Adrenalin as a sympathetic stimulant could probably indirectly play an important rôle by setting up visceral perturbations, thereby initiating afferent impulses and increasing central "tonus." There is much evidence that augmented central activity generally results in raising the tonus of skeletal muscle. This topic is more fully discussed in a paper now in publication elsewhere (10).

Hoskins (11) in discussing the effect of adrenalin in augmenting the activity of skeletal muscle suggested that the vasodilator effect accounts, at least in part, for its effect upon this tissue. However, in discussing adrenalin and muscular activity Gruber (2) points out that "epinephrin can produce vasodilatation in the muscle vessels only when the nerves are intact, or, after nerve section, only when some time has elapsed" and that he has "never been able to get vasodilatation of muscle vessels immediately after cutting the nerves leading to the muscle." Nevertheless, under such conditions, the augmenting effect can still be demonstrated.

There remains yet another possibility whereby adrenalin may affect the

action of skeletal muscle. Boothby and Sandiford (12) report that when adrenalin is injected into dogs in amounts which are comparable with that secreted by the adrenal glands basal metabolism in all or nearly all types of cells is increased. To what extent, however, this might lead to heightened tissue reactivity and thus greater muscular tonus without increase of stimulation is not clear.

In discussing the direct action of adrenalin upon skeletal muscle Gruber states that the elimination of fatigue products is one of the ways in which this drug betters muscular contraction, but presumably this would not significantly affect tonus, at least under ordinary conditions.

At the present time the mechanism whereby adrenalin affects skeletal muscle remains undetermined.

During the past year the influence of various factors on neuromuscular activity as exhibited by the knee-jerk has been under investigation in this laboratory. The effects of adrenalin upon this reflex have been obtained in graphic form and are presented in this paper.

The data presented were collected from nine normal subjects in good health. Our group was necessarily limited due to the difficulty in getting subjects to submit to the inconvenience and sometimes discomfort of intramuscular injection of this drug. The considerable demand on the time of the subjects was also a deterrent.

The data were collected by means of an especially constructed apparatus described in detail elsewhere (13). In the experiment herein reported the frequency of stimuli was 7 per minute.

It was desirable to use a dose of adrenalin which would produce at least some of the effects obtained by other investigators. The response to subcutaneous injections of this drug was first brought to attention by Eppinger and Hess (14) who found that in Graves' disease 1 mgm. of adrenalin caused a marked rise in blood pressure, tachycardia, mydriasis, tremor, palpitation and subjective nervous disturbances, while in normal subjects there was little or no response. Goetsch (15) reported that such effects were elicitable in this disease by a dose of only 0.5 mgm. subcutaneously administered. That those effects are not confined to pathological cases is shown by Clough (16) who reports that in studying subjects with hypertension he found that 18 per cent of the normal group responded markedly, while 82 per cent responded slightly or moderately to 1 mgm. of adrenalin. Tompkins, Sturgis and Wearn (17) reported that the intramuscular injection of 0.5 cc. adrenalin, 1:1000, caused an increase in metabolic rate and in the respiratory quotient in case of normal persons. In treating Addison's disease Rowntree (18) reported a marked improvement on giving 1 cc. of adrenalin, 1:1000, by rectum three times daily, together with 5 minims hypodermically twice daily and 10 grams of whole gland by mouth three times daily.

In consideration of the effects produced in both normal and pathological cases it would seem that from 0.5 to 1.0 cc. of adrenalin 1:1000 should be sufficient to cause a change in the knee-jerk if this reflex is influenced at all by this drug. The general procedure in this experiment was to use 0.5 cc. of adrenalin 1:1000 in case the subject had taken none previously. The largest dose given to any subject was 1.8 cc. of the 1:1000 solution of adrenalin chloride (Parke, Davis).

The usual method of procedure was to allow each subject to deliver normal kicks for some minutes, after which adrenalin was given in the doses indicated.

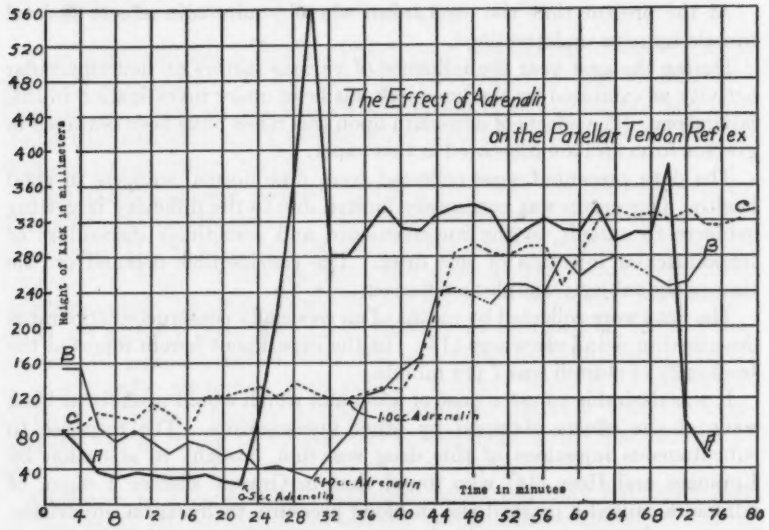


Fig. 1. This figure shows the effect of adrenalin upon the patellar tendon reflex.

Subject 1 is a man of thirty-one years who is well trained in knee-jerk experiments. This, however, was his first experience in taking adrenalin. The effect is shown in figure 1. After delivering normal kicks for twenty-two minutes the subject was given 0.5 cc. adrenalin. Immediately following the injection the jerk was augmented for a little more than a minute (*B*, fig. 1), after which an apparently normal record was registered for a little more than a minute (*A*, fig. 1). At the end of this time the extent of the knee-jerk began to increase, reaching a level which it maintained for forty-eight minutes. At the end of this time it returned to normal (*X*, fig. 1), remaining thus until the end of the experiment.

In a little more than two minutes after the dose was given the subject reported that he felt dizzy. This was soon followed by partial blindness

and deafness which lasted for about ten minutes. These effects were accompanied by nausea which lasted well through the experiment. Soon after the dose was given tremor was noticed, beginning in the hands and extending over the whole body. Respiration became slow and deep, the subject throwing his head backward during inhalation and forward during exhalation. The skin became pale and covered with perspiration. The skin felt cold and clammy to the operator. The musculature of the whole body became tense. The following day the subject reported that he had experienced a severe headache which came on soon after the experiment ended, lasting for three or four hours. Upon subsequent occasions this subject took two doses of adrenalin by mouth, 3 cc. and 5 cc. respectively. Each time nausea, tremor and headache followed, indicating that he is unusually susceptible to this drug.

Subject 2 is a man of twenty-two years who has had experience in knee-jerk experiments. He had taken three 1.0 cc. doses of adrenalin previous to this experiment. After delivering normal kicks for thirty minutes he was given 1.0 cc. of adrenalin intramuscularly. There was no augmentation of the jerk at the time the dose was given. Two minutes later the extent of the jerk began to increase, reaching a level which it maintained throughout the experiment, which lasted forty-four minutes. This subject experienced effects similar to those of subject 1 but much less marked. The first effect was tremor, which was most pronounced in the hands. The respiration became somewhat dyspneic. All visible parts of the body were pale and the subject reported that the musculature of his entire body felt tense. These effects lasted until the subject ate his dinner, which was about three hours after the experiment. This subject reported that the effects of adrenalin upon previous occasions were similar to those experienced in this experiment.

Subject 3 is a man of thirty-five years who is experienced in knee-jerk experiments. He had taken three 1.0 cc. doses of adrenalin previous to this experiment. After delivering normal kicks for thirty-four minutes he was given 1.0 cc. of adrenalin intramuscularly. The knee-jerk was not augmented at the time the dose was given. Six minutes later the extent of the reaction began to increase, reaching a level in eight minutes, which it maintained throughout the remainder of the experiment. This subject reported that his musculature felt tense and that he was unsteady. He experienced tremor, which was most noticeable in the hands. All visible parts of the body became pale. The pulse was strong and rapid. These effects were very noticeable for three or four hours after the experiment. He also reported that his previous experiences in taking adrenalin were very similar to those in this experiment.

Subject 4 is a man of twenty-seven years. He had had no experience in knee-jerk experiments. After delivering normal kicks for twenty-eight

minutes he was given 0.5 cc. of adrenalin. The experimental period lasted forty-six minutes. The knee-jerk was not affected by either the needle or the dose during this time and the subject reported that he experienced no effects from the adrenalin.

Subject 5 is a man of forty-three years. He was well trained in knee-jerk experiments and had taken several doses of adrenalin. After delivering normal kicks for thirty-three minutes he was given 1.0 cc. of adrenalin intramuscularly. During the course of thirty-one minutes no change in the extent of the knee-jerk was recorded.

On another occasion, after delivering normal kicks for twenty-three minutes this subject was given 1.8 cc. of adrenalin. During the course of thirty minutes the knee-jerk remained unaffected. The stimulation due to inserting the hypodermic needle did not affect the knee-jerk in either experiment. The only effect noted was a slight tremor of the hands.

Subject 6 is a man of thirty-eight years. He had had some experience in knee-jerk experiments, but had not taken adrenalin previously. After delivering normal kicks for twenty-two minutes he was given 0.6 cc. of adrenalin. The experiment was terminated in twenty-four minutes. The record showed no change in the extent of the knee-jerk during this time. The subject reported that he felt no effects from the drug during the experiment. Some time after the record was made a very slight tremor of the hands was noted.

Subject 7 is a man of twenty-five years who has had a great deal of experience in knee-jerk experiments. He had never taken adrenalin previous to this experiment. After delivering normal kicks for thirty-seven minutes he was given 0.5 cc. During the course of twenty-five minutes the knee-jerk was not affected. Upon another occasion this subject was allowed to deliver normal kicks for twenty-six minutes, after which he was given 1.0 cc. of adrenalin. The experimental period lasted fifty-two minutes. During this time the knee-jerk was not affected by the adrenalin. The subject reported that he experienced no effects due to the adrenalin during the course of the experiment. However, a short time after each experiment tremor in the hands was quite noticeable.

Subject 8 is a man of forty-five years who had had some experience in knee-jerk experiments. Previous to this experiment he had taken several doses of adrenalin. After delivering normal kicks for thirty-one minutes he was given 0.5 cc. of the drug. The reflex was augmented for two minutes at the time of the injection, after which it returned to normal. The experiment was terminated in forty minutes. During this time the knee-jerk was not affected by the adrenalin. The subject reported that neither on this nor on any previous occasion did he experience any effects due to this drug.

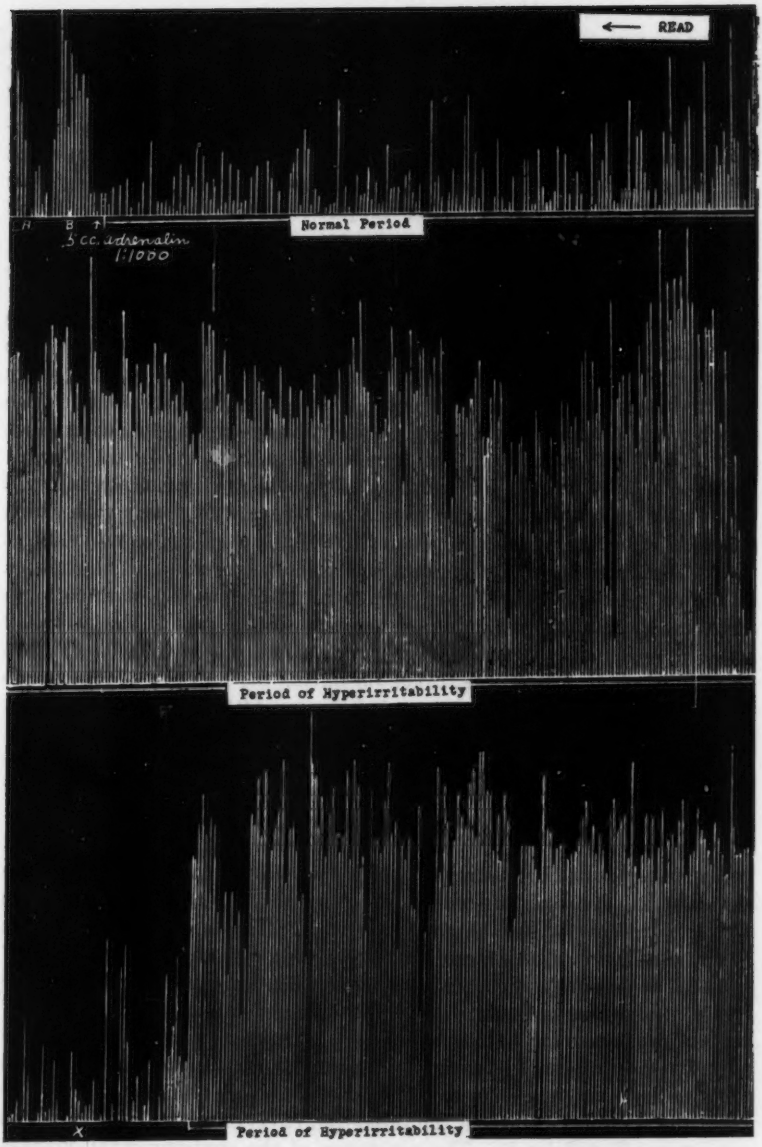


Fig. 2. The effect of adrenalin upon the patellar tendon reflex. *aa*—subject 1; *bb*—subject 2; *cc*—subject 3.

Subject 9 is a man of twenty-four years. He had had experience in knee-jerk experiments but had not taken adrenalin previously. After delivering normal kicks for twenty-seven minutes he was given 0.8 cc. of adrenalin. The experimental period lasted fifty-four minutes. Immediately following insertion of the needle the knee-kicks were augmented for two minutes, after which they returned to normal, remaining thus throughout the experiment. This subject reported that he experienced no effects due to adrenalin.

Figure 2 shows curves plotted from the data obtained from subjects 1, 2 and 3. These were the only subjects affected by adrenalin.

Use is made of the well-known smoothing device in order to eliminate irregularities which are characteristic of knee-jerk records. In this case one-minute averages were plotted. "Height of kick in millimeters" refers to the distance through which the stylus moved on the kymograph. The relation of the "height of the kick" to the distance through which the foot moved is as one is to four. This correction has been made, however, in constructing the graph. The force of the stimuli was purposely adjusted to give rather low initial kicks because of the greater delicacy with which the effect of experimental factors can thus be detected. In case of subject 1 (*AA*, fig. 2) the period of stimulation due to the needle and the return to normal (*A* and *B*, fig. 1) are submerged in the process of smoothing. The latent period after the dose of adrenalin in the case of the subject 2 (*BB*, fig. 2) is not shown by the curve for the same reason. Due to the fact that the electric current was turned off during the progress of the experiment of subject 2 there are two gaps in his curve (*BB*, fig. 2) lasting eleven and three minutes respectively.

Discussion. It has been demonstrated by Lombard (19) and corroborated in this laboratory that the knee-jerk may be materially affected by sensory stimulation of various kinds. A number of situations are constantly encountered in the laboratory which cannot be controlled. A considerable amount of stimulation is necessarily caused by the intramuscular injection of adrenalin. Whether the subject anticipated the action of the drug must also be considered. The question then arises: just what effect did these factors have on the data presented in this paper?

As far as possible the experimental conditions were kept constant. The ordinary disturbances such as the passing of vehicles, the changing of classes from one room to another and the occasional entrance of an unexpected visitor could not be avoided. These disturbances were irregular and brief, as were the responses to them. Reënforcement due to such stimuli are easily distinguished from the prolonged effect of adrenalin in case the subject is affected by it. The constancy of the environmental changes makes reënforcement caused by such factors neglible for they appear in the control as well as in the experimental period. Reënforcement due

to outside factors has been submerged by smoothing in graphs presented. It is the opinion of the writer that environmental changes have not significantly influenced these data.

Stimulation due to the intramuscular injections as such could not be avoided. Of the nine cases, subjects 1, 8 and 9 alone showed the effect of this stimulation on their records. Negative results in the other eight cases were probably due to intentional "passivity," i.e., anticipatory inhibition. Subject 1 was the only member of this group who reacted to the adrenalin. However, after the period of hyperactivity due to inserting the needle the reflex returned to normal, remaining thus for more than a minute before any of the effects of adrenalin were observed. The knee-jerk of subjects 8 and 9 returned to normal two minutes after the dose was given and remained thus throughout the experimental period.

The effect of suggestion upon the knee-jerk is well known and must be considered in all experiments upon this reflex. In all but one case the subjects were well trained. Of the susceptible group, subject 1 had no knowledge of the effects likely to be produced by adrenalin; subjects 2 and 3 had had experience with this drug on other occasions but had no preconceived notion as to whether the knee-jerk would be augmented, depressed or unaffected. Subjects 2 and 3 reported that the general reactions produced on other occasions were quite similar to those induced in this experiment. The fact that none of the subjects knew what to expect eliminates anticipation as an influencing factor.

Of the group studied, three experienced an augmentation of the knee-jerk and six were unaffected. Of the subjects used only those who experienced tenseness of muscles, marked tremor, nervousness, etc., gave augmented knee-jerks.

The question arises whether they were normal or pathological. From the personal history of these subjects nothing could be detected which would indicate that any of the diseases affecting the action of adrenalin were ever present.

It seems most probable that the adrenalin causes a change in tonus of skeletal muscle which is brought about directly by stimulation of sympathetic fibers, providing the dose is adequate. Further data, however, are obviously necessary to determine the point. It is planned to continue the study on animals instead of human subjects, no more of whom are available for this particular experiment.

SUMMARY

Adrenalin chloride was administered intramuscularly to nine human subjects in doses of from 0.5 to 1.8 mgm. In three cases the knee-jerk was markedly augmented; in six, unaffected. In the three positive cases various symptoms indicated high sensitivity to adrenalin. Augmentation

XUM

of the knee-jerk following administration of adrenalin is possibly due to a direct effect of the drug on the sympathetic fibers to the skeletal muscle.

It is a pleasure to acknowledge my indebtedness to Prof. R. G. Hoskins who directed these experiments. I also wish to thank Mr. George Woodward for his assistance throughout this work.

BIBLIOGRAPHY

(1) CANNON AND NICE: This Journal, 1914, xxxiv, 243.
(2) GRUBER: Endocrinol., 1919, iii, 145.
(3) HOSKINS AND DURANT: This Journal, 1923, xlvi, 177.
(4) PERRONCITO: Arch. ital. de biol., xxxviii, 393 (Quoted by HUNTER: Med. Journ. Australia, 1924, i, 86).
(5) BOEKE: Anat. Anz. 1913, xliv, 343.
(6) DE BOER: Folia neurol., 1913, vii, 378, 837.
(7) LANGELAAN: Brain, 1915, xxxviii, 235.
(8) LANGELAAN: Brain, 1922, xlv, 435.
(9) ADRIAN: Med. Sci., 1920, ii, 454.
(10) TUTTLE: Journ. Exper. Psychol. (in press).
(11) HOSKINS: Endocrinol., 1917, i, 292.
(12) BOOTHBY AND SANDIFORD: This Journal, 1923, lxvi, 93.
(13) TUTTLE: This Journal, 1924, lxviii, 338.
(14) EPPINGER AND HESS: Zeitschr. f. klin. Med., 1909, lxvii, 345; lxviii, 205 (Quoted from CLOUGH: Johns Hopkins Hosp. Bull., 1920, xxxi, 266).
(15) GOETSCH: New York State Journ. Med., 1918, 259 (Quoted from CLOUGH: Johns Hopkins Hosp. Bull., 1920, xxxi, 266).
(16) CLOUGH: Johns Hopkins Hosp. Bull., 1920, xxxi, 266.
(17) TOMPKINS, STURGIS AND WEARN: Arch. Int. Med., 1919, xxiv, 269.
(18) ROWNTREE: Med. Clin. N. Amer., 1923, vii, 177, 187.
(19) LOMBARD: Amer. Journ. Psychol., 1887, i, 5.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 69 AUGUST 1, 1924 No. 3

THE EFFECT OF CHEMICAL COMPOUNDS ON THE PRODUCTION OF THE TETANY SYNDROME

W. W. SWINGLE AND J. S. NICHOLAS

From the Zoölogical Laboratory, Yale University and the School of Medicine, University of Pittsburgh

Received for publication April 24, 1924

Many workers have claimed that reactions similar to those resulting from parathyroid extirpation can be secured in certain mammals following administration by one method or another, of various chemical substances. Thus Berkeley and Beebe (2) have secured muscular twitching by intravenous injections of ammonia, which, taken in conjunction with the work of Macallum and Voegtlin (9) who observed that the urine content of ammonia increases in parathyroidectomized dogs, would seem to indicate that ammonia has some significance in the reaction of the animal to parathyroid tetany. Carlson and Jacobson (4), however, find that the increase in ammonia content of the blood of parathyroidectomized dogs falls well within the limit of experimental error. Moreover, Wilson, Stearns and Thurlow (13) have shown that the initial condition during parathyroid tetany is one of low ammonia output and it is only after the onset of tremors and convulsions that the ammonia output is increased.

Paton and Findlay (10) quote Bostock as having found a persistence of tremor after ammonia injection during anesthesia, at which time the tremors of parathyroid tetany are completely suppressed. They have also mentioned their own observations, which seem to show that the very nature of the increased tremor is primarily different. There is a fine tremor after parathyroid extirpation, a coarse, jerky tremor after ammonia injection. The question of ammonia injection as a method of producing parathyroid symptoms would, therefore, seem to be pretty well settled.

Berkeley and Beebe (2) were able to cause convulsions, snapping of jaws and muscle twitchings by the intravenous injection of xanthin. Their results could not be repeated by Voegtlin and Macallum using the same dosage and method of administration.